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HENRY VAN PETERS WILSON
1863–1939

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HENRY VAN PETERS WILSON

Memorial Resolution

Adopted by the Faculty of The University of North Carolina

In the passing of Dr. H. V. Wilson on January 4, 1939, the University has lost one of the ablest teachers it has had, the faculty one of the wisest and most thoughtful counselors, the students one of the most generally esteemed and respected instructors, and colleagues, students and members of the community generally a stimulating companion and a highly cherished friend.

Henry Van Peters Wilson was born in Baltimore, Maryland, February 16, 1863; educated in the public schools, including the Baltimore City College, where, to his enduring satisfaction, he had rigorous training in the classics, and then in the undergraduate division of The Johns Hopkins University.

After brief experiences in teaching, and in studies at a Medical School he reëntered The Johns Hopkins University to start upon the professional career from which there was thereafter no deviation. As a graduate student at Hopkins in the department made famous by Martin and Brooks, he was associated with a select group of exceptionally able young men, several of whom have since acquired high distinction in scientific research. Even before completing his graduate work, Wilson had acquired the particular respect and admiration of those around him for his notably thorough and precise research and for his wide reading and accurate analyses of literature and of observations.

Having obtained the degree of Doctor of Philosophy from Hopkins, Dr. Wilson held the Bruce fellowship of that institution for a year and

then served for two years as scientific assistant in the United States Fish Commission at the historic Fisheries Biological Laboratory at Woods Hole, Massachusetts. In this position he did not lose himself in administrative or routine duties or scatter his energies widely over practical fishery problems. On the contrary he undertook and carried to completion a masterly study of the embryology of the sea bass, the results of which were published in a monograph which has proved to be much more than a thorough meritorious piece of research; it has, indeed, frequently been used as a working model for graduate students.

In 1891 Dr. Wilson assumed his first, and one might say his last, regular teaching position when he was brought to Chapel Hill as head of a one-man Department of Biology. The only changes of title that came to him in the remaining forty-seven years of his career in the University were when the Department, after substantial growth under his guidance, was divided into Departments of Botany and Zoology, Dr. Wilson continuing as head of the Department of Zoology, when again in 1917 he was made Kenan Professor, and finally when, at his own request in 1935, he was relieved of administrative duties as head of the Department of Zoology. He continued as Kenan Professor, actively engaged in teaching, until the time of his death.

Almost his whole professional career was thus associated with this University and his success in teaching and in the administration of the Department and his achievements in research have played no insignificant part in establishing and spreading the reputation of the University of North Carolina for thoroughness and soundness of scholarship. When the young professor assumed the responsibilities of a Department of Biology in the University at Chapel Hill, the enrollment of students in the institution was less than 250, the revenues of the University were extremely restricted and the departmental budget was at a minimum. From the very beginning, Dr. Wilson determined that the Department of Zoology should not only be thorough in its work, but relatively broad in its offering and as well-equipped as the possibilities would permit. The number of courses he taught well and the amount of work he accomplished seem now remarkable. He offered courses in elementary physiology, in general biology, comparative anatomy or mammalian anatomy, embryology, medical histology, microtechnique and invertebrate morphology, besides occasional courses in general botany and systematic zoology. He accomplished all of this efficiently and continued to be productive in research only by intense concentration and the most systematic distribution of time and effort. He assumed also

a most meticulous habit with respect to the care and preservation of apparatus and material. In after years some friends have smiled at his regular habit of counting corks and vials and his measured dealing of cover glasses, pins and tacks, but it was through unfailing adherence to practices of economy that within a relatively short time the Department of Zoology came to have a body of equipment comparable to that of many institutions where the departmental budgets were two or three times as large.

Dr. Wilson's teaching, research and developmental activities were not restricted to Chapel Hill. Very early he began the practice of spending the greater part or all of his summers in the little seaport of Beaufort, North Carolina, already made famous in the biological world through the studies in marine biology that had been carried on there by Agassiz, Gill, Coues, Morse, Brooks and others. Through Dr. Wilson's influence the United States Fish Commission was soon led to establish a summer laboratory at Beaufort, and he became its first Director. The laboratory was conducted at first in a rented residence on the water-front and then for several summers in a leased warehouse adjoining the town wharf. One of Dr. Wilson's most esteemed colleagues, Professor J. A. Holmes, then State Geologist and lecturer in geology in the University, joined with him heartily and most effectively in the effort to put the United States Fisheries Biological Laboratory upon a permanent basis. So it was that in 1902 the first permanent marine biological laboratory south of New England came into being on a small island that had been presented to the Government by friends of Dr. Wilson, and with a building and plant largely as planned by him. The Laboratory, which continues in operation to this time, has been the temporary home of a great number of prominent zoologists, botanists and physiologists, who have found it a favorable place for the conduct of original research. It has also played a significant part in the exploration of fishery problems in the State and in the entire southeastern region. The fathers of this Laboratory were unquestionably Henry Van Peters Wilson, as the initiator and original director, and Joseph A. Holmes, whose practical vision and adeptness in public relations encompassed and solved the practical problems incident to its establishment.

It was in the Beaufort Laboratory that Dr. Wilson began most of his important research work, obtaining the material that was studied in spare hours and week-ends during the following winters in Chapel Hill. On a few occasions, while on leave, Dr. Wilson worked at other laboratories, notably Berlin and Naples, but by far the greater and the

more significant part of his research was done at Chapel Hill and Beaufort. Perhaps the most brilliant work accomplished by Dr. Wilson at these places was that dealing with the dissociation of cells and regeneration, a discovery and a technique which attracted world-wide attention and which lie at the very foundation of modern tissue-culture and experimental embryology. Even apart from this and the related researches which followed, Dr. Wilson would have won a permanent place in the annals of biological science for his studies of embryology and of the morphology, systematics and embryology of sponges.

Dr. Wilson was a member of the National Academy of Sciences; the Philosophical Society of America; the American Society of Zoologists, which he served as President in 1911; the Boston Society of Natural History; The American Association for the Advancement of Science (fellow); Société Linnéenne de Lyon; the Elisha Mitchell Scientific Society of which he was President in 1905–06 and again in the last year of his life; and the North Carolina Academy of Science of which he was President in 1912; he was representative of the American Society of Zoologists in the National Research Council 1929–32; and rendered editorial service at various times to the Journal of Morphology, Biological Abstracts and the Journal of the Elisha Mitchell Scientific Society.

It would not be appropriate here to catalogue his contributions to science nor would we attempt a complete analysis of his characteristics. We would, however, record our recognition and appreciation of some of the personal qualities which made him so effective as a teacher and so helpful to the institution. Dr. Wilson was notable for his extremely systematic habits, his great capacity for work, his clarity of thought and his lucidity and incisiveness in speech. He possessed a most highly developed critical faculty associated with his unyielding devotion to accuracy and to the best standards of life and thought and expression. He had a strong personal interest in good students and a lasting affection for them, which, it need scarcely be said, was reciprocated. Lacking patience with slovenliness of dress, speech, work, or thought, Dr. Wilson preserved inflexibly his high ideals for himself, for his students and for the University. Always courageous in expression of opinion, he never hesitated to condemn what he thought worthy of condemnation or to stand in the minority, or even alone, for that which seemed to correspond with his belief as to what was best. He was, indeed, no insignificant factor in making the University what it is today.

We have enjoyed his companionship, we have respected and admired his strength of character, his depth of conviction, his devotion to high

academic and personal standards. We mourn his passing, which leaves a distinct gap in our ranks. We extend our deepest sympathy to his children and to his sister and we felicitate them upon the memory of a father and brother who was master of his circumstances, distinguished in accomplishment and an exemplifier of the best in personal ideals.

WILLIAM DEB. MACNIDER,
FRANCIS F. BRADSHAW,
ROBERT E. COKER,
Committee for the Faculty.

SUPPLEMENTAL BIBLIOGRAPHY

There will be found in Volume 50 (1934) of this JOURNAL a list of Dr. Wilson's publications, 71 titles, while connected with the University from 1891 to 1933. In the following list are comprised: (1) His publications prior to 1891; (2) Certain omissions from the original bibliography; and (3) The titles of publications subsequent to 1933.

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1887. The Structure of *Cunoctantha octonaria* in the Adult and Larval Stages. Stud. Biol. Lab. J. Hopk. Univ. **4**: 95–107, 3 pls.
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1888. Abstract of Observation on the Development of *Manicina areolata*. J. Hopk. Univ. Circ. **7**: 31–33, 2 cuts. Abst. J. R. Micr. Soc. 1888: 434–435; Amer. Nat. **22**: 355.
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1889. On the Occasional Presence of a Mouth and Anus in Actinozoa. J. Hopk. Univ. Circ. **8**, no. 70: 37–38. Abst. J. R. Micr. Soc. 1889, pt. 6: 761.
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1892. Non-Sexual Reproduction in Sponges. Amer. Nat. **26**: 626–628. Abst. J. Morph. **5**: 511–519.
1931. What Has Made the University of North Carolina What It Is? (Privately printed July; reprinted September)

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1934. Cell Behavior in the Metamorphosis of the Halichondrine Sponge Larvae. Abst. J. Elisha Mitchell Sci. Soc. **50**: 50.
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1937. Notes on the Cultivation and Growth of Sponges from Reduction Bodies, Dissociated Cells, and Larvae. *In* Galtsoff: Culture Methods for Invertebrate Animals: 137–139.

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THE MORPHOLOGICAL AND CYTOLOGICAL DEVELOPMENT OF THE SPOROPHYLLS AND SEED OF JUNIPERUS VIRGINIANA L.*

By Andrew Clark Mathews

Plates 1–9

I. HISTORICAL INTRODUCTION

Despite the fact that considerable investigation has been made on the morphological and cytological features of the more widespread species of the sub-family Cupressineae of the family Pinaceae, a complete and thoroughgoing story embracing both morphology and cytology does not exist. Since the life history of the eastern North American species, *Juniperus virginiana* L., is so little known, a treatment of this kind for this species is highly desirable. The inclusion of a morphological study of the small berry-like ovulate cone of this species is also particularly worthwhile in view of the fact that the homologization of the cone structure of the Cupressineae with that of the Abietineae has been a matter of considerable discussion among plant morphologists for a long period of time.

In 1907, Norén (47) gave a thorough historical account of the cytological work that had been done on *Juniperus* from the time of Hofmeister and Strasburger. Norén worked out the complete cytological story of *Juniperus communis*, including micro- and mega-sporogenesis, fertilization and the early elongation of the prosuspensor tier of the proembryo. Although Hofmeister (1851; 1858) made a few cytological studies of *J. communis* and *J. sibirica*, and Strasburger (1872, etc.) studied parts of the story of *J. virginiana*, no complete and detailed account with modern techniques had been made before Norén.

In 1909, Alice M. Ottley (48) published a cytological study of *Juniperus communis* and *J. virginiana*, and in 1910, Nichols (45) gave a cytological account of *J. communis* var. *depressa*. The major portion of Miss Ottley's study was on *J. communis*. Nichol's work was a

* A thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany.

consideration of the American variety of *J. communis* and was very thorough. He went into more detail in regard to pollen tube development and especially the division of the body cell than Norén but to a lesser extent into megasporogenesis.

In 1933, Karl and Hally J. Sax (54) reported chromosome counts of representative conifers, including *J. virginiana*. The materials used for their study were chiefly the female gametophyte tissue and pollen mother cells in the two meiotic divisions of the latter.

No full account of the embryogeny of *Juniperus* has appeared since Strasburger, and his investigation which included *J. communis* and *J. virginiana* cannot be considered as entirely complete. Saxton and Buchholz have presented the greater part of our further knowledge of the embryogeny of the Cupressineae. Saxton (58) included a rather full account of this particular phase in the life history of *Actinostrobus pyramidalis* Miq. in 1913, while Buchholz presented the details of the embryogeny of *Biota* (*Thuja*) and *Libocedrus decurrens* in 1926 (7) and *Chamaecyparis obtusa* in 1932 (9).

Among the recent and outstanding contributions bearing on the morphological features dealt with in the present paper, Quisumbing's (1925) investigation of the seeds of conifers included a study of the stony layer development of *Juniperus virginiana*. Dluhosch (1937) studied *J. chinensis* in connection with his work on the development of the microsporophyll of the conifers.

The question as to whether the female cone of conifers is to be regarded as a simple or compound floral structure has been a subject of considerable discussion among morphologists for a long time. The studies and discussions that have been made of this question since 1827, in which year Robert Brown announced gymnospermy, are presented by Coulter and Chamberlain (1932, pp. 244–251). In 1926, Pilger (49) presented a thorough treatment of this question in his morphological account of the conifers in Engler-Prantl's *Pflanzenfamilien*, pp. 124–155. Since 1926, Lanfer (1933), Hagerup (1933; 1934), Hirmer (1936) and Propach-Gieseler (1936) have published rather exhaustive treatments of this subject. In all recent accounts, especially those of Hirmer and Propach-Gieseler, a comparative morphological study of the female cone-scale has been paramount. Miss Propach-Gieseler's paper presents a very detailed study of the earliest developmental stages of the cones of the Cupressineae, including several varieties of *Juniperus virginiana*, and her paper is, therefore, of great importance on this point.

According to Mohr (43), *J. virginiana* is native to eastern North America, extending along the Atlantic seaboard from Maine to Florida and westward to Minnesota and South Dakota on the north, and south to the vicinity of Austin, Texas, and the Gulf of Mexico. This species is exceptionally valuable for its timber, for wildlife food and protection, and for ornamental purposes.

II. MATERIALS AND METHODS

Juniperus virginiana is a dioecious conifer, and in order to obtain all the developmental stages in its life history, it was necessary to collect the female material from a site where male and female trees were growing close together. A clump of trees of both sexes was found near the campus of Duke University, Durham, N. C., and the majority of the female material for this study was collected there during the spring and summer of 1933. Slabs were cut from two sides of the flowers and developing cones with a razor blade and the material plunged immediately into the fixing fluid. Collections were made every two days beginning just prior to time of pollination, viz. about March 1st, until archegonial formation, about May 25th, and once a day and sometimes more often until after fertilization, viz. June 8th. The remainder of the female material was collected from trees in the Coker Arboretum of the University of North Carolina and from other places in Chapel Hill, N. C.

The male material was collected from trees on "Windy Hill" in Chapel Hill, N. C., and also prepared for fixation by cutting slabs from two sides of the cones. The collecting was begun in the fall of 1933, beginning about August 1st and repeated about every two or three days until September 30th. By this time the pollen grains were formed and only an occasional collection was made thereafter until about the time of pollination the following spring.

Several fixing fluids and modifications of them were used. The following formula, a modification of that suggested by Chamberlain (15, p. 343), served as a good general one for the female material:

A. 0.5 c.c. chromic acid
   3.0 c.c. glacial acetic acid
   100.0 c.c. distilled water
B. Add 1% osmic acid in proportion of one part to 15 parts of A at the time of fixation.

The hot alcoholic corrosive sublimate—acetic acid recommended by Miss Ethel Thomas (Chamberlain, 15, p. 343) gave unsatisfactory re-

sults with the late stages of the female gametophyte. The fixing fluids found most satisfactory for the male cones were the chrome-osmo-acetic acid solution recommended by Miss Ferguson (25) and the Chicago chromo-acetic-osmic solution of Chamberlain (15). The majority of the embryogeny material was fixed in formalin-alcohol as recommended by Buchholz (6). However, a part of the material used in the study of embryogeny was sectioned in paraffin.

Haidenhain's iron-alum haematoxylin with orange G as a counter-stain gave excellent results. In tracing the development of starch in the pollen mother-cell cytoplasm as well as the fate of the starch brought into the egg cytoplasm by the sperm cell, a special staining combination of the safranine (alcoholic) and gentian violet (aqueous) of Flemming's Triple plus a very strong iodine solution was found very helpful. The slides were taken through safranine and left about 10 minutes in gentian violet. Then, after two dips in water, all excess water was removed from around the sections, and the strong iodine solution dropped on and allowed to remain for 3–4 minutes. The iodine was then removed and the slide dehydrated as quickly as possible by dipping in 95% alcohol and absolute alcohol, dipped about twice in clove oil and then into xylol for clearing, and mounted in balsam.

In staining whole sections of mature berry-cones, excellent differentiation was obtained by staining overnight in alcoholic safranine, destaining with acidulated alcohol, and completing with Delafield's haematoxylin for about 20 minutes. As recommended by Buchholz (7), dissected proembryos and embryos were stained in Delafield's haematoxylin and mounted in diaphane.

III. THE STAMINATE CONE AND THE MICROSPOROPHYLL

In his recent paper on the development of the microsporophyll of the conifers, Dluhosch (22) has given a brief review of the history of our knowledge of the development of the microsporophyll of conifers. In 1845, H. von Mohl considered the male conifer cone as a single flower, bearing sporophylls each of which bears two or more sporangia. Parlatore (1868) regarded single sporophylls as flowers, and his view predominated until the work of Celakowsky (1897). Celakowsky's view was that the primary form of the conifer sporophyll is radiate as in *Taxus*, and that the pollen-bearing leaf has been transformed from the shield-radial form to the bilateral form, in which only the lower side of the leaf has remained fertile, or pollen-sac bearing. Goebel's (1923) view agreed essentially with that of Celakowsky. He held that

the microsporophylls of *Taxus* and the Cupressineae are shield-shaped, and in spite of varying conditions within single groups and even within single flowers, that this may be extended to all species of conifers.

Dluhosch (22) agrees with Goebel in regarding the primary structural form of the conifer microsporophyll to be shield-shaped and the origin of the sporangia as marginal. He also describes for the Cupressineae the subsequent development of a sterile outgrowth just above the sporangial primordia, which is more or less marked in the different genera. This outgrowth together with the distal sterile tissue of the original sporophyll ultimately gives rise to a sort of "false" or secondary shield or peltate form. Dluhosch also reports the occasional occurrence of two rows of sporangia on the fertile shield margin in *Chamaecyparis* and *Thuja*. In these genera, the median portion of the primitive primordium of the sporogenous tissue becomes sterile, and the two rows of microsporangia are differentiated out of the initials of the resulting sporogenous tissue.

At Chapel Hill, N. C., the staminate cones of *Juniperus virginiana* become recognizable early in August (Figs. 1, 2) before pollination and fertilization the following spring and summer. The microsporangia pass the winter filled with mature microspores (Figs. 11, 36). Figure 3 is an outline of a longitudinal section of a staminate cone of *J. virginiana* at the time when the microsporangia contain mature pollen mother-cells, and Fig. 4 shows a mature cone just before pollination. The mature cone is made up of four opposing rows of 3 to 4 shield-shaped sporophylls on the median cone-axis. Miss Ottley (48) reported that at Wellesley, Massachusetts, the microsporangia of *J. virginiana* pass the winter in the pollen mother-cell stage, and that the microsporangia of *J. communis* do not begin development until the spring of the season of pollination. Nichols (45) also found that the staminate cones of *J. communis* var. *depressa* at Hartford, Connecticut, are first recognizable in late summer and pass the winter in a more or less rudimentary condition.

In Chapel Hill, the development of the microsporangia of *J. virginiana* is accomplished in a little less than two months, viz. from early in August until about the latter part of September (Figs. 5–11; 13; 14). Fig. 5 shows a very young potential microsporophyll at the top of a young male bud (as Fig. 1) prior to the bud's being recognizable as a male cone. In this potential sporophyll the resin cavity has already appeared, and after examination of several of these very young cavities it was concluded that they originate schizogenously. This agrees with Haberlandt's (26, p. 516 and p. 522) conclusion concerning the resin

cavities of *J. communis*. A striking characteristic of the sporophyll as well as vegetative leaves of *J. virginiana* is the median resin canal in the abaxial side of the sporophyll just beneath the epidermis. This canal runs parallel to the long axis of the leaf, extending from near the tip of the leaf and ending blindly just above the resin cavity. The canal seems to be formed by a mere separation of the epidermal layer from the mesophyll layer. The mesophyll cells are very loosely arranged, containing a large quantity of intercellular air space between them. The majority of these cells are richly supplied with chloroplasts, but some are decidedly resinous in character, although some cells seem to contain both chloroplasts and resin. It was observed that these chloroplasts become filled with large starch grains before the end of summer as evidenced in part by Figs. 10 and 11.

In a very short time the sporangial primordia become recognizable on the abaxial side and basal end of the young microsporophyll (Fig. 6). This is shown by the outward projection of the sporophyll tissue at this point; and just beneath the epidermis the primary archesporial cells are recognizable by their larger size, larger nuclei, and denser cytoplasm. The archesporium represented by Fig. 6 consisted of three cells in a plate parallel to the epidermis. Meanwhile, the resin cavity has enlarged considerably, and the sporophyll has grown a little longer. The epidermal cells also begin to acquire some resin. This resin deposit persists during the remainder of the development.

Nichols (45) has reviewed the history of our knowledge regarding the origin of the microsporangium of many gymnosperms. He shows that Goebel (1881), Coulter and Chamberlain (18), and other investigators, notably Coker (16) and Coulter and Land (21), have shown that the development of the microsporangium in gymnosperms is essentially similar to that of the eusporangiate pteridophytes. *Juniperus virginiana* is in agreement with this latter view.

It is now easy to follow the further development of the microsporophyll (Figs. 7–9; 12). Norén (47) did not consider the origin and early development of the microsporophyll and microsporangia in the European *Juniperus communis*; Nichols (45) described in considerable detail the microsporangial development in *J. communis* var. *depressa* but not the early morphological development of the microsporophyll of this species. However, Dluhosch (22) included *J. chinensis* in his morphological treatment of the microsporophylls of the conifers. *Juniperus virginiana* agrees in all essential respects with Nichol's description of the sporangial development and that of Dluhosch on the development

of the microsporophyll of *J. chinensis*. In *J. virginiana* the microsporangium continues to grow and project outward, and very soon a second outgrowth of the sporophyll becomes recognizable distally with reference to the young sporangia and immediately adjacent to them (Fig. 7). This outgrowth early becomes somewhat flattened and tapered at its outermost point and continues its downward growth in close association with the microsporangia (Figs. 8, 9). It finally becomes the sterile shield-half of the mature peltate sporophyll (Fig. 12).

During this early evagination of the sporophyll-outgrowth, the plate of archesporial cells within the microsporangium has divided periclinally and anticlinally to produce more archesporial tissue centrally located and surrounded by a sub-epidermal layer of sterile sporogenous cells. By periclinal division of this sub-epidermal layer and concomitant divisions of the sporogenous cells the three sporangial wall layers shown in Fig. 8 result. The primary sporogenous cells which are ultimately formed become thus surrounded by a one-layered tapetum which is recognizable by its uniformity and dense cytoplasm, an intermediate layer of tabular cells, and the outer epidermal layer. The sporangial wall is thus considered to be comprised of two wall layers. This differs from the sporangial wall of *Pinus*, which has been shown by Miss Ferguson (25), Mathews (40) and others to consist of 3 to 5 layers of cells. Fig. 8 also shows that the sporophyll out-growth has elongated still further downward and around the sporangia. The construction of a sporangium just prior to meiosis of the pollen mother-cells is shown in Fig. 9.

IV. MEIOSIS OF THE POLLEN MOTHER-CELL AND FATE OF THE MICROSPORANGIAL WALL LAYERS

It is difficult to say how much time is consumed in the meiotic divisions of the pollen mother-cells to produce the tetrads of microspores, for different male cones on the same day may be in different stages of this process. The cones at the end of a given branchlet are not usually so far advanced in meiosis as those farther back from the end. Nichols (45) reports that this process is completed within a week in *J. communis* var. *depressa* and Norén (47) and Miss Ottley (48) did not report the time required. It would seem that the meiotic divisions of the pollen mother-cells in *J. virginiana* are accomplished in approximately a period of 2–4 days at Chapel Hill, N. C. The sequence of the meiotic activity is made easier to follow due to the fact that in the earliest stages the meiotic division is farther along in sporangia at the base of the cone

than at the cone apex; however, during the latter part of meiosis, several cases were noted in which this sequence seemed to have been reversed so that the more advanced stages of meiosis appeared toward the apical end of the cone.

Miss Ottley (48) did not go into the details of meiosis in *J. virginiana*, but Norén (47) and Nichols (45) made thorough studies of this in *J. communis*. The essentials of meiosis in *J. virginiana* are in close agreement with those described by these authors for *J. communis*; and as also borne out in part by these authors, the nuclear behavior at this time bears a striking similarity to that described by Allen (1) for *Lilium canadense*, Mottier (44) for *Podophyllum*, *Lilium*, etc., and Miss Ferguson (25) for *Pinus*. The mature pollen mother-cells are polyhedral, nearly isodiametric and approximately 20 microns in diameter (Figs. 9, 15, 38). The nuclei of the pollen mother-cells are about 10.5 microns in diameter and usually contain two conspicuous nucleoli. The measurements for *J. virginiana* are approximately 2 microns less than Norén (47) and Nichols (45) reported for *J. communis*; and the nuclei are only about one-third as large as those of *Larix* and *Pinus*. The chromatin material presents a finely reticulated appearance with small chromatin granules rather irregularly arranged along the linin threads. At some places two threads seem to lie beside one another as if beginning to show signs of pairing (Fig. 15), but this could not be positively demonstrated. Just prior to synizesis the chromatin threads have become quite conspicuously lumpy, and begin to be drawn in away from the nuclear membrane. Pairing of the chromatin threads is more readily discernible at this stage (Fig. 16 (1), 16 (2)). The threads continue to draw in more closely until they form a tight ball that usually lies at one side of the nuclear cavity. Figs. 17, 18, and 19 show progressive stages in the behavior of the nuclear material after synizesis. Nichols stated for the chromatin material at this point in *J. communis* that from observations made at the time of synizesis and from preceding and subsequent phenomena, "It may be inferred that there is present at first a series of double threads, and that toward the close of synapsis these coalesce and unite end to end to form a single bivalent spireme." This appears to be the case in *J. virginiana*. Gradually the spireme becomes transversely segmented; and the segments begin to shorten, thicken and become somewhat twisted (Figs. 20, 21). By this time, the nuclear membrane is no longer a definite thin membrane, but merely a wide band of dense fibrils which fade out into the surrounding cytoplasm. Fig. 22 represents a stage subsequent to the diakinesis stage of

Sharp (61). The chromatin segments are completely differentiated just prior to becoming arranged at the equator of the heterotypic spindle.

By this time the pollen mother-cells have lost their angular shape and are considerably more spherical and separated from one another. Nichols suggests that this is apparently brought about by the dissolution of the middle lamellae of the mother-cells in the manner described by Strasburger (1882).

Gradually the weft of fibers derived from the nuclear membrane together with fibers derived from the cytoplasm push their way into the nuclear cavity and constitute the spindle for the heterotypic division. Figs. 23 and 24 show the meta- and telophases of the heterotypic division. Upon arriving at the poles the chromosomes very soon become enclosed by a delicate nuclear membrane and begin to show considerable vacuolation and become somewhat irregularly distributed throughout the nuclear cavity. The mature resting daughter nuclei of the division are generally biconvex with the long axis perpendicular to the axis of the meiotic spindle (Figs. 25, 26). As pointed out by Coker (16) for *Taxodium*, Norén (47) for *J. communis*, and Mathews (40) for *Pinus palustris*, there appears to be a temporary cell plate laid down at the equator between the daughter nuclei of this division.

After a brief rest period the daughter nuclei of the heterotypic division undergo the second meiotic or homotypic division (Figs. 27, 28, 31). The two nuclear spindles may be variously oriented in the cell, but as noted by Nichols (45), tetrahedral arrangement seems to be more prevalent. The chromosomes are generally "V" or "U" shaped, short and thick, and somewhat irregular in outline. The cell plates laid down after this division are at first similar to that of the heterotypic division, but they gradually become thicker and are persistent (Figs. 28, 31). Gradually a deposit of what appears to be a mucilaginous layer begins to be laid down at the periphery of each of the four newly formed microspores of the tetrad. As noted by Norén (47) and Nichols (45), *Juniperus* does not possess as definite and rigid-appearing walls as Miss Ferguson (25) has described for *Pinus*. The fact that mucilaginous walls do exist is evidenced, however, by the microspores being definitely separated from one another by a narrow interval (Fig. 32); but these walls, if they may be considered as such, take orange G stain only to a slight extent, and they are of very short duration, since the general liberation of the microspores takes place very soon.

The other investigators of *Juniperus* have noted the difficulty of obtaining the chromosome count in meiosis. Norén (47) considered the

haploid number in *J. communis* to be eleven, while Nichols (45) was convinced after considerable study that the number in var. *depressa* was twelve. Karl and Hally J. Sax (54), working at the Arnold Arboretum, made chromosome counts for numerous conifers, including *J. virginiana*, *J. communis*, and *J. chinensis*. They used the aceto-carmine smear technique on mitoses of female gametophytic tissue and meiosis of pollen mother-cells. A resumé of their work shows the basic haploid number of chromosomes to be twelve for most gymnosperms, except the Gnetales. Exceptions to this fact, however, were found in the Cupressineae and Taxodineae in which the prevailing number was eleven, in *Pseudotsuga* thirteen and *Pseudolarix* twenty-two. According to these authors, "deviations from the primary basic number of 12 are attributed to the loss of a small chromosome, following translocation of segments, in the Cupressineae and Taxodineae; duplication of a chromosome in *Pseudotsuga*; and polyploidy in three species (including a variety of *Juniperus chinensis*) of Conifers." Some effort was made by the writer to obtain an accurate chromosome count in *J. virginiana* from polar views of the anaphase stage in the meiotic divisions of the pollen mother-cell. As accurate a count as was obtainable showed the haploid number to be 11 (Figs. 29, 30), which checks with the studies made by Sax and Sax.

As suggested by Mathews (40) for *Pinus palustris*, the liberation of the microspores from the tetrad seems to be effected also in *J. virginiana* by a dissolution (probably enzymatic) of both tetrad chamber walls and the old mother-cell wall which surround the tetrad (Fig. 33). The microspores have no developing air-sacs to effect a mechanical rupture as described by Miss Ferguson (25) for *Pinus*, and the general appearance of the liberation seems to be one of chemical dissolution. When the young microspores are liberated, they have the general shape of their tetrad chambers, are delimited by a delicate pliable membrane and possess one rather large resting nucleus which is at first devoid of a nucleolus (Figs. 33, 34). The microspore cytoplasm contains a rich supply of starch which in haematoxylin preparations gives it a marked frothy appearance. Very soon, however, the microspore begins to round itself off, and the thick exine and thinner intine walls begin to be laid down about the periphery of the plasma membrane (Fig. 35). The young liberated microspores are about 12μ in diameter, while their nuclei measure approximately 6.5μ. Before the beginning of the winter period of dormancy, the young microspore has grown to about 16.5μ, and the starch grains which it contains have enlarged considerably.

Meanwhile, the intine and exine walls become thicker. While the microspore becomes larger, its nucleus becomes smaller as it assumes its resting condition for the winter, its average size at this time being about 5 microns. Its chromatin threads have arranged themselves into a finely reticulated structure and the nucleus contains one definite nucleolus. There is no division of the microspore nucleus before pollination.

Particularly worthy of note is the development of starch in the cytoplasm of the pollen mother-cell. The investigators of *J. communis* have described the presence of starch in the developing tetrad of microspores, but their figures do not entirely agree with the condition in *J. virginiana*. It would seem unlikely that there should be much difference in these two species in this respect. Using a combination of Flemming Triple stain and strong iodine solution, the presence of starch was easily detected in the mother-cell cytoplasm at the different stages. (See figures of meiosis, Plate 2, especially Figs. 38–40.) The starch grains are small just before synizesis in the mother-cell, but they become rapidly larger, and at the close of the heterotypic division they are quite large and show a rather characteristic alternate arrangement above and below the temporary (ephemeral) cell plate (Figs. 25, 40). As noted by Nichols (45), throughout the entire development of the microspore tetrad the cytoplast shows a striking "alveolar" character. This is obviously due to its rich starch content. The starch grains of the young pollen grain are very large and occupy the major portion of the cell (Fig. 36). Each starch grain has two or more lamellae and a rather distinct hilum. This stage is found near the end of the growing season, namely, late in September. By the time of pollination the following spring, starch no longer is apparent in the cytoplasm of the pollen grain (Fig. 37). This is in accord with the investigation of J. Doyle and Clinch (24), who studied certain seasonal changes in leaves of *J. virginiana* and other conifers.

During meiosis of the pollen mother-cell and development of the young pollen grain, several changes have occurred concomitantly in the microsporangial wall. Figs. 9, 10, and 11 show the successive changes as they occur in the tapetum and inner and outer wall layers from the mature pollen mother-cell stage until the sporangium is filled with young pollen grains. The outer wall layer, which is epidermal in origin, and the tapetum contain numerous starch grains at the end of the heterotypic division of the pollen mother-cell and are the only prominent layers surrounding the mother-cells. The cells of the inner wall layer

have already become considerably stretched and crushed and are rapidly degenerating. By the end of September the majority of these cells have been completely absorbed and only an occasional remnant remains (Fig. 11). Meanwhile, only a few starch grains show up in the outer sporangial layer. At this stage, the tapetum contains very little starch, and its cells have begun to appear considerably degenerated. Just before pollination the following spring, the inner sporangial wall layer and tapetum have been completely adsorbed, and only the outer wall layer remains to constitute the microsporangial wall. The cells of this layer are rectangular with their long axes parallel to the long axis of the sporophyll stalk cells, and their internal walls show prominent cellulose thickenings (Figs. 12–14). These thickenings appear as strong bands which are seen in side view to alternate on either side of the cell extending from the top of the cell downward laterally to the mid-line at the bottom of the cell, thereby furnishing considerable strength to the sporangial wall and functioning in its dehiscence.

V. POLLINATION

Pollination in *Juniperus virginiana* occurs between the middle and last of February in the vicinity of Chapel Hill. The spring season was early at Chapel Hill in 1938, and pollination had been mostly completed on the trees of this species in the Coker Arboretum by February 10th, but had not begun on the trees at "Windy Hill" on this date.

The majority of the male cones bear sixteen sporophylls (Fig. 4), each sporophyll bearing 3-4 pendent sporangia on its lower surface. The microsporangial wall splits longitudinally on the lower side to discharge the pollen grains. This dehiscence is the mere separation of the rectangular sporangial wall cells in a line parallel to their longitudinal axes. Consequently, the line of dehiscence is parallel to the longitudinal axis of the sporophyll. When a ripe sporangium is pricked with a needle, there occurs a rather active sporangial dehiscence, and as the pollen grains are discharged, the edges of the sporangial wall curve backward from the point of rupture. It is very likely that the internal cellulose bands of the sporangial wall cells (Figs. 13, 14) play a large part in the natural dehiscence by being drawn when dry toward the mid-line of the cell (Fig. 14) and thereby creating considerable tension on the sporangial wall. These cellulose bands would seem, then, to serve in the same capacity as the thickened portions of the annular cells of the leptosporangiate fern sporangium.

As has been noted by other investigators, the micropyle of the ovule

is wide open at the time of pollination (Fig. 63), and, as probably first recorded by Jack (30) for *J. communis*, "from each orifice there is exuded a minute globule of clear, shining liquid which rests like an iridescent bubble on the tip and serves to catch the pollen and conduct it to the nucellus or ovule within." Lawson (37) also noted that this pollination-drop is observed more frequently during the early morning, before the heat of the day has caused evaporation. The pollen grains finally lodge in the saucer-like depression at the tip of the nucellus, and after a few days the micropyle is closed due to the elongation of the layer of integument cells lining the micropyle. These cells elongate in a plane transverse to the long axis of the ovule and are generally known as micropyle-closing cells (Fig. 82a). In paraffin sections made just after pollination, the pollination-drop shows at the nucellar tip as a flat, wrinkled mass, apparently of a mucilaginous nature (Fig. 82a).

VI. THE MALE GAMETOPHYTE

Early Development.—The history of our knowledge of the pollen tube of *Juniperus* begins with Hofmeister's (28) investigations of *J. communis*. He found that two years are required for the pollen tube development in this species, and at the time of fertilization he saw a large cell in division in the end of the tube and a great many small cells (about 10 are figured) above this one. Hofmeister (29) published another account of the pollen tube development and fertilization in *Juniperus* in 1858. This time he observed the division of the large cell of the pollen tube into two smaller cells (the sperm cells). According to his interpretation, these two cells produced by repeated divisions sixteen free cells which then passed over the archegonia, or four medium-sized or eight smaller spherical cells without very definite membranes, which probably arose from repeated divisions of a still larger initial spherical cell. However, Strasburger (65) was able to find only granular cytoplasm in the pollen tube of *J. communis*. In 1872, though, Strasburger (66) found in *J. virginiana* a large free cell which divided into two daughter cells before fertilization. In 1878, the same author (67) gave another and more accurate account of the pollen tube of *Juniperus*, but this time he seems to have misinterpreted the contents of two tubes as the contents of one. Belajeff (4) was the first to show the correct interpretation of this phase of development. He found that the young pollen tube divided into two dissimilar cells, and that the cell remaining behind in the pollen tube divided further into a cell and a free nucleus, the first preceding the second as they both moved

forward into the tube. In the tube, this cell was overtaken by the free nucleus with the result that a mobile group consisting of a cell and two nuclei was formed at the anterior end of the pollen tube. Finally, he observed that the cell of this mobile group later divided into two equal sperm cells, both of which might participate in fertilization.

During the winter rest period before pollination in *J. virginiana* the pollen grains have completely lost their starch content, their cytoplasm now being left filled with numerous large vacuoles, and their cell walls, especially the exine, having become much thicker (Fig. 37). The pollen grain nucleus is very definitely spherical, occupies a central position in the cell, and in no instance was observed to divide before pollination. The pollen grains are wingless, as is true of those of all other Cupressineae. Lawson (37) has shown that in the Cupressineae, with the exception of *Cupressus* and *Juniperus*, the first division of the microspore nucleus occurs before pollination, giving rise to the generative and tube nuclei. He also shows that the Cupressineae are characterized by the absence of vestigial prothallial cells or of nuclei representing such cells.

In *J. virginiana*, pollination and fertilization take place during the same growing season, a little less than four months apart. This species agrees with most gymnosperms in this respect, but differs from *J. communis* (Norén, 47; Ottley, 48; Nichols, 45) and *Pinus* (Ferguson, 25; Mathews, 40) in that fertilization follows the next season after pollination in these species.

According to Nichols (45), the division of the primary pollen grain nucleus in *J. communis* to form the generative nucleus and pollen tube nucleus occurs within a week after the pollen grain reaches the tip of the nucellus, the generative nucleus becoming surrounded immediately by dense cytoplasm and separated from the "pollen cell" by a thin plasma membrane. In *J. virginiana*, very soon after this division, the exine wall ruptures and the intine wall is pushed outward to accomplish germination. Fig. 41 shows a pollen grain at the tip of the nucellus; and stages of germination nearly a month later are shown in Figs. 42 and 43. In these stages the lenticular generative cell is still in the extreme pollen-grain-end of the young pollen tube, while the pollen tube nucleus has begun to migrate downward in the tube. In Fig. 42, the pollination-drop is shown collapsed at the tip of the nucellus. In the section showing the pollen tube of Fig. 43, the ruptured exine wall was located just above the pollen grain end of the tube. Near the base of the nucellus the megaspore mother-cell is at this time in its resting stage or in a very early stage of its meiosis (Figs. 82a, 82b).

The pollen tube now begins to push its tip between the cells at the nucellar tip; meanwhile the generative cell enlarges and rounds up, and the tube nucleus moves nearer the end of the tube (Fig. 44). Some of the nucellar cells are crushed and the protoplasts of nearly all the cells immediately adjacent to the tube become permanently affected, if not destroyed. All the young pollen tubes observed showed the pollen-grain-end of the tube somewhat protruded above the nucellar tip (Fig. 44). The elongation of the young tube while it is within the pollination-drop and before it reaches the nucellus may be the explanation of this condition. In *J. communis*, the developing male gametophyte passes the winter with tube contents nearly similar to those shown for *J. virginiana* in Fig. 44.

While the spherical generative cell is still in the pollen-grain-end of the tube, its nucleus divides to give rise to the body cell nucleus and stalk cell nucleus. In *J. communis*, Nichols (45) shows that these two nuclei, of which the body cell nucleus is the smaller, at first lie free in the cytoplasm of the tube, while the body cell nucleus soon becomes invested with a zone of dense cytoplasm which develops a membrane, and that a true stalk cell is never formed. While this division was not observed in *J. virginiana*, a body cell with a dense cytoplast and only a stalk cell nucleus result from the division of the generative cell. These promptly move down the pollen tube and come to lie near the tube nucleus a short distance from the tip of the tube (Fig. 45). Each of the three nuclei of the pollen tube has a single well-defined nucleolus and the chromatin material is evident in each nucleus as a very delicate reticulum. The stalk nucleus becomes closely associated with the pollen tube nucleus very soon after being formed, and it is and usually remains smaller than the latter. The pollen tube elongates only to about one-third the length of the entire nucellar cap when it ceases further elongation for approximately a month. Fig. 46 shows the contents of a pollen tube which had penetrated about one-third of the nucellar cap and was about a month older than that shown in Fig. 45. The body cell nucleus has grown nearly twice its former size, and the cell has acquired a considerably larger cytoplast. Thus it is in the 3-celled stage in *J. virginiana* and not the 2-celled stage as in *J. communis* that the pause in the elongation of the pollen tube occurs. Accompanying the increase in size of the body cell, its cytoplasm gradually becomes denser and the cytoplasmic granules become larger. Later developmental stages reveal the numerous granules contained in the body cell cytoplast at this time to be starch. Since the pollen tube cytoplasm is entirely devoid of starch and the nucellar cells are abundantly supplied

with it, it is very likely that the subsequent enlargement of these granules before fertilization is due to the translocation of starch from the nucellar cells to the body cell cytoplasm.

During this pause in the elongation of the pollen tube, the megaspore mother-cell at the base of the nucellus undergoes meiosis and the functional megaspore gives rise to the massive free-nucleate megagametophyte, which in turn becomes cellular and the cellular megagametophyte ultimately bears a group of very young archegonia, before any further marked elongation of the pollen tube occurs.

During the latter part of May, the pollen tube again becomes active and forces its way through the nucellar tissue, crushing and disorganizing the cells of the nucellus with which it comes in contact and within a few days enters the archegonial chamber where its tip presses down close to the necks of the archegonia (Fig. 47). By this time, the body cell, which usually becomes somewhat elongated during its downward migration, begins to round up and finally to become quite spherical after it comes to rest just above the stalk and pollen tube nuclei in the end of the tube. Its cytoplast is now very dense and surrounded by a thick plasma membrane.

Division of the Body Cell.—Miss Ferguson (25) described the cytology of the body cell mitosis in *Pinus*, showing that there are formed two unequal sperm nuclei both occupying a common cytoplast in which also both stalk cell and pollen tube nucleus may or may not be included. The larger sperm nucleus is, as a rule, in advance of the other in the pollen tube. Coker (16), working on *Taxodium*, was the first to give an accurate description of the cytology of the division of the body cell for a species which behaves similarly to *Juniperus*. Nichols (45) treated this point in considerable detail for *J. communis*. This author stated that the body cell of *J. communis* divides about four days after the pollen tube enters the archegonial chamber and about three days before fertilization. Nichols also showed for *J. communis*, as did Coker (16) for *Taxodium*, that this division in the species studied usually occurs simultaneously with that of the central cell of the archegonium. Two equal cells are usually formed in these species, and like the body cell, each is bounded by a definite membrane (Fig. 48). Nichols showed for *J. communis* that these cells are at first hemispherical and lie close together, but after separating become approximately spherical. In *J. virginiana* they usually lie side by side as if the spindle of the body cell mitosis had been perpendicular to the long axis of the pollen tube (Fig. 48). The average diameters of the body cell and sperm cell are

approximately 57 microns and 44 microns, respectively. A mature sperm and a fragment of its mate are shown in Fig. 49. The nucleus has a thick membrane, a coarse reticulum of linin threads which bear irregular deposits of chromatin, and usually one rather large and well defined nucleolus. Like the body cell, the sperm cytoplast contains a dense aggregation of minute starch grains, but the cytoplasm does not show a radiate structure as described by Coker (16) for *Taxodium* and Norén (47) for *J. communis*. This feature of *J. virginiana* is also indicated for *J. communis* by Nichols.

Contrary to the type of spindle described by Miss Ferguson (25) for the body cell of *Pinus*, which is extranuclear and unipolar, Nichols (45) shows that in *J. communis* spindle fibers "originate entirely within the nucleus." Other details pointed out by Nichols are: 1. The body cell usually loses its spherical shape as it approaches division and becomes ovoid. 2. There is present within the nucleus a delicate protoplasmic network in addition to the chromatin and linin proper. 3. The coarse, anastomosing reticulum, in which chromatin cannot well be distinguished from linin, resolves itself into a slender, uniformly distributed spireme which shows beautifully a distinction between linin and chromatin; and the chromatin granules are very clearly arranged in pairs at fairly regular intervals along the lighter staining linin band. 4. The spireme segments into the haploid number of slender and often twisted chromosomes; simultaneously delicate granular fibers arise within the nucleus. 6. The chromosomes rapidly become shorter and thicker, soon orienting themselves at the equatorial plane where the fibers give rise to a blunt, multipolar diarch spindle; the nuclear membrane disappears.

The production of more than two male cells (or the functional equivalent of two male cells) by a single pollen tube has been described for several species of gymnosperms, including some of the Cupressineae. Some phylogenetic speculations have also been based on this point. To give a complete account of this matter from our present knowledge of the Cupressineae, we find that the majority of this sub-family seems to show striking agreement with *Taxodium* (Coker, 16) and *Cryptomeria* (Lawson, 36) of the Taxodineae in bearing normally only two equal male cells in the pollen tube. A striking similarity also exists in the general shape and character of the male cells in these plant groups. These facts have been borne out in the following species and genera: *Thuja* (Land, 32; Lawson, 37), *Libocedrus decurrens*, *Cupressus* (two species), and *Chamaecyparis* (Lawson, 37), *Juniperus communis* (Norén,

47; Ottley, 48; Nichols, 45), *J. virginiana* (Ottley, 48), *Widdringtonia cupressoides* (Saxton, 56), *Callitris* (Saxton, 57), *Actinostrobus pyramidalis* (Saxton, 58), and *Tetraclinis articulata* (Saxton, 59). The writer has found no indication of the presence of more than the two male cells in the tube of *Juniperus virginiana*.

In spite of the fact that two male cells in a single pollen tube seems to be the rule in the Cupressineae, Norén, Nichols, and Saxton have reported exceptional cases in which more than the usual two sexual cells and two sterile nuclei were present in the tube. Norén (47) described the occurrence of a large body cell with three nuclei in the pollen tube of *Juniperus communis*; Nichols (45) found several cases in which more than two male cells had been formed by the division of the body cell in the tube of *J. communis* var. *depressa*; and Saxton (57) reported a case in *Callitris verrucosa* in which "a large nucleus of quite a different character" was present in the pollen tube in addition to the usual contents. While these cases can only be considered as very exceptional, Juel (31) found several male cells (as many as twenty) in a single pollen tube to be the rule in *Cupressus goveniana*, and Doak (23) reported a complex of several male cells in most cases in *C. arizonica*. (Doak also states that the male cells of *C. arizonica* have a distinctive pear-like shape suggestive of motility.) On the other hand, Lawson (37) found that in two other species of *Cupressus* examined by him more than two male cells are never formed in a single pollen tube. Apparently the only other cases among the investigated gymnosperms in which the production of more than two male cells by the body cell has been reported are *Microcycas* (Caldwell, 11), in which the body cell regularly produces sixteen or more sperms; and *Ceratozamia* (Chamberlain, 14), in which four sperms are formed occasionally.

VII. MORPHOLOGICAL DEVELOPMENT OF THE OVULATE STROBILUS

The female flowers of *Juniperus virginiana* are first recognizable about the middle of September before pollination and fertilization take place the following spring. They are borne terminally on short axillary shoots from small branches produced during the current season (Figs. 50a, 50b). The female sporophylls are not recognizable as such until a few weeks subsequent to pollination, namely about the last of March; but at least two pairs of the female sporophylls grow up over and gradually coalesce above the one or two ovules to form the dark blue berry-like fruit. (The blue color of the berry-cone is normally obscured by a gray "bloom" which covers its surface.)

Pilger (49), in *Die Natürlichen Pflanzenfamilien*, simply states regarding the structure of the berry-cone that (1) the cones of the Sabina Section of the Juniperoideae are as a rule formed from six scales and (2) that the berry-cones of *J. virginiana* contain 1-2 seeds. In view of the fact that Miss Charlotte Propach-Gieseler (51) has very recently published an account of the developmental details of the female flowers of the Cupressineae, including four varieties of *J. virginiana*, it was deemed desirable to compare the species treated in the present paper with this author's results.

The majority of the female flowers of *J. virginiana* are composed of a very short axis bearing two pairs of opposing sporophylls which are designated from the cone apex downward as pair *a* and pair *b*. Exceptions to this are: (1) The growing-point of the shoot may extend high enough above the usual level to produce an additional pair of sporophylls, pair *a'*; and (2) the pair of scale-like leaves below pair *b* often becomes fleshy in berry-cones bearing two ovules and fuses with the sporophylls above to construct the ultimate berry-cone. Invariably, however, sporophyll pair *b* alone shows fertility, usually only one member being fertile but sometimes both members. A more extensive study into the gross morphology of the female flowers of this species shows, furthermore, that there are three well-defined flower-types. These are treated here under types A, B, and C. Flower-type A: This flower-type contains only one ovule which is borne in the axil of one member of the sporophyll pair *b*, designated sporophyll *b̲*. Approximately two-thirds of the female flowers are of this type. Figs. 51 and 52 show the general structure of this flower-type, while Figs. 55 and 56 are diagrammatic transverse sections of the flowers shown in Figs. 51 and 52, respectively. Flower-type B: Approximately one-half of the remaining one-third of female flowers bear two ovules side by side in the axil of only one member of sporophyll pair *b*, while the other sporophyll of the pair is sterile. This type is represented in Figs. 53a and 53b; Figs. 57 and 58 are diagrammatic transverse sections of this flower-type. Flower-type C: The remaining one-sixth of the female flowers are of this type and are characterized by each member of sporophyll pair *b* being fertile and bearing a single ovule in its axil. Figs. 54 and 59 show the gross structure of this flower-type. The fertile sporophyll (or sporophylls) is never constant as to its circumferential position on the cone-bearing branch in all three of these flower-types. Furthermore, while no flowers of *J. virginiana* were found to bear more than two ovules, Miss Propach-Gieseler (51) describes the closely re-

lated species, *J. sabina*, as having 1–4 ovules with one member of the sporophyll pair *b* bearing only one ovule in the 3-ovuled flower and each member of this pair bearing two ovules in the 4-ovuled flower. In *J. keteleri*, whose flowers bear 2–6 ovules, the sporophyll pair *c* may be fertile.

In the maturation of the berry-cone, an outgrowth develops on the morphological upper side of the cone-scale and is termed "fruit-scale" by Hirmer (27) and Miss Propach-Gieseler (51), while that portion below the fruit-scale bearing the "point" is termed "cover-scale" by these authors. It is due largely to the profuse up-growth of the fruit-scales and their final coalescence above that the ovule (or ovules) becomes entirely enveloped in the mature berry-cone. The sporophyll-outgrowth of the fertile sporophyll contributes considerably more to the fleshy tissue of the berry-cone than its sterile neighbors. Figs. 60a, 60b and 60c represent different views of a mature berry-cone developed from Flower-type A described above. The dotted line indicates the outline of the seed. In this type the cover-scale tip of fertile sporophyll *b* is solitary on one side of the fruit, and the seed is borne in its axil. The cover-scale tips of the three sterile sporophylls are in a group directly on the opposite side of the berry-cone. A mature berry-cone produced from the Flower-type B is represented in Fig. 61. Here the arrangement of the cone-scales is principally the same as in Flower-type A, except that the fertile sporophyll *b* bears two seeds in its axil and the sterile sporophyll pair *c* usually enters into the construction of the berry-cone. The berry-cone resulting from the Flower-type C is different in shape from the other two types, being somewhat flattened on the sides of sporophyll pairs *a* and *c* and in general obovoid, whereas the other types are usually simply ovoid (Fig. 62). In the fruit of flower-type C, the sporophyll members of the pairs *a*, *b*, and *c*, which usually coalesce to form the fleshy cone of the fruit, directly oppose each other morphologically as do the vegetative leaves immediately below. Each member of sporophyll pair *b* bears one seed in its axil, and sporophyll pair *c*, as in Type B, takes a very minor part in the constitution of the fleshy fruit coat.

As the epidermal cells of the opposing sporophyll-outgrowths (fruit-scales) come in contact with each other above the ovule, they become somewhat elongated in the direction of the opposing fruit-scale. The final fusion is accomplished by an alternate interlocking of these epidermal cells of opposing sporophylls in a manner somewhat similar to the enmeshing of two cogwheels (Fig. 68). All the epidermal cells of

the sporophyll tissue are cutinized, but those of the epidermis outside the berry-cone have a thicker cuticle than those at the point of fusion and those lining the inside of the sporophyll coat.

VIII. NOTES ON THE COMPARATIVE MORPHOLOGY OF THE OVULATE STROBILUS

The ovulate cone of *Juniperus* differs from that of other Cupressineae in the coalescence of its fruit-scales around and above the ovule, or ovules, to form a solid and simple berry-like cone. Miss Propach-Gieseler (51) has shown that the development of all the Cupressinean female cones is essentially similar. Fertile scale-pairs usually occupy a rather central position on the cone-axis, above and below which lie sterile scale-pairs, no sterile pair occurring between fertile scale-pairs. It may be noted from Figs. 51–62 above that in *J. virginiana* sporophyll whorl (pair) *b* is fertile as a rule, while whorls *a* and *c* are sterile. The ovule grows out of a meristematic tissue which lies in the lower part of the morphological upper side of the fertile sporophyll. Usually the ovule is formed at the base of the megasporophyll which bears it.

As described above, the fertile cone-scales of *J. virginiana* as well as the normal sterile ones are 2-parted, consisting of the cover-scale and fruit-scale (Figs. 63–66). According to Hirmer and Propach-Gieseler, this is true of all the conifers. Hirmer regards the cover- and fruit-scales of conifers as arising by a serial splitting of the primitive megasporophyll primordium. While the cover- and fruit-scales of *J. virginiana* appear as a single unified organ (the cone-scale), the fruit-scale at the time of pollination appears as a slight bulge on the adaxial side of the cone-scale (Fig. 63). Even at this early stage the fruit-scale and cover-scale are each supplied with a separate vascular bundle, and this condition persists throughout the development of the cone (Figs. 63–66; 79).

Miss Propach-Gieseler (51) has shown that the Cupressinean cone closes its apical development with a sterile leaf structure, for which she suggests the use of the term "Akrokon." This term is taken from the shape of the fertile shoot which is narrowly conical and which together with the upper scale-pair forms the akrokon. In the older stages, however, the growing-point becomes broad and flattened, and the last-formed scales have extended over it and so closely drawn together at their bases that the growing-point is no longer distinguishable; the uppermost pair of scales alone represents the akrokon.

The same author has shown furthermore that the mature ovulate

cones of *Juniperus* do not differ essentially from those of other Cupressineae. The number of ovules of the section Sabina varies from 1–6. In *J. virginiana* there is usually only one ovule which is located at the base of (and adaxial to) a scale of whorl *b*; but because of an upward extension which takes place in the scale-pair *a* at the top of the shoot, this pair is pushed to the summit of the cone. The three ovules of *J. communis* are not borne in the upper megasporophyll whorl in whose axils they stand, but opposite the joints of the lower scale whorl (*b*), which agrees with the condition in the other genera. Here as elsewhere, except in *Cupressus goveniana*, the uppermost scale-whorl (*a*) represents the akrokon.

It is beyond the scope of the present paper to enter into a discussion of the interpretation of the floral-type (viz. simple flower vs. compound inflorescence, etc.) of the ovulate cone of *J. virginiana*. Gymnosperm morphologists seem fairly generally agreed that the male conifer cone represents a simple flower structure; but the female cone with its 2-parted cone-scales has been more of a problem to them. Pilger (49) has compiled a comprehensive account of the older theories bearing on this point. But among the recent investigators and theorizers are Lanfer (33), Hagerup (1933, 1934; see 34), Hirmer (27), and Propach-Gieseler (51). The last named conducted her detailed morphological study of the Cupressineae under the direction of Hirmer. Suffice it to say here that with very exhaustive investigations on which to base their interpretations, Lanfer and Hagerup adhere to the view that the female conifer cone is a compound inflorescence, while Hirmer regards it as a simple female flower. The papers of Hirmer and Propach-Gieseler are remarkable for their profuse illustrations, including photomicrographs and diagrams of the early development of cones of representative groups and a considerable amount of comparative morphological data. Hirmer traces the origin of the typical conifer cone and sporophyll unit back to the fossil lycopod-like Sphenophylls. In the opinion of this author, the single elements of this flower have split serially, thereby cutting off an adaxial (fruit-scale) and an abaxial (cover-scale) segment. Both of these segments are supplied by a separate vascular strand, but only the adaxial segment is ever fertile and bears a megasporangium or megasporangia. This fundamental plan, according to Hirmer, exists throughout the conifers, and he points out that it is not difficult to understand how by lateral fusions of the vegetative (abaxial) segments of all the sporophyll units of a cone, accompanied by certain concomitant changes

of the fertile (adaxial) segments internally the angiospermous ovary may have had its phylogenetic origin.

IX. THE STONY LAYER

Coulter and Chamberlain (20) say concerning the developing seeds of the Pinaceae that "the integument differentiates into . . . three layers . . . ; but the outer fleshy layer does not deserve its name, for it is represented only by a thin layer of cells that disappears with the maturing of the testa. The middle or stony layer in this case is the conspicuous one, the seed being said to ripen dry; while the inner fleshy layer is most largely and distinctly developed, as usual, in the free portion of the integument." Conforming to this description, the integument of *J. virginiana* differentiates itself into three layers. The middle stony layer has a very striking mode of development and is a very prominent part of the mature seed. It is also primarily due to the development of the stony layer that the integument finally becomes differentiated clearly into its three layers. Perhaps the most outstanding paper treating this subject is Quisumbing's (52) comprehensive study of the stony layer of seeds of gymnosperms, which includes the seed of *Juniperus*. The following account is deemed a worthwhile addition to Quisumbing's description.

About May 1st, when the ovule contains a free-nucleate megagametophyte at about the 64-nucleate stage, the secondary thickening which characterizes the stony layer first becomes recognizable in the micropyle-closing cells above the nucellar tip (Fig. 70). Quisumbing (52) states that this secondary thickening first shows up as late as the early appearance of the archegonial initials. The cell marked "X" in Fig. 70 is about the first of the middle layer of cells below the micropyle-closing layer to show noticeable secondary thickening. As was noted by Quisumbing, the order of progress of this secondary thickening of the integument cells continues downward through the middle region of the integument from the micropyle-closing layer. This can be traced in the diagrams and camera lucida figures 66, 67, 69, 70, 71a, and 79. Gradually, as the cells lower down the integument begin to show secondary thickening, the number of the integument cells which is to give rise to the inner papery layer increases. Sections of early stages in stone cell formation show the secondary deposit to be laid down on the inner surface of the thin primary wall to form a distinctly reticulated pattern. The phloroglucin and hydrochloric acid test shows this deposit to be

lignin. As the lignification of this middle integument tissue continues downward around the ovule, the deposit higher up in the young stony layer gradually becomes thicker. The larger spaces in the reticulation become smaller and smaller as more lignin is deposited (Figs. 71a, 71b, 72–78), so that finally only small orifices remain open to the interior of the well-defined pits in the thick stone-cell wall (Figs. 75–78). The cytoplasts of these stone-cells apparently remain quite normal and active throughout the entire lignification period, and indeed, their nuclei are still conspicuous in mature stone-cells of berry-cones collected August 5 (Figs. 77, 78, and 80). It is not unusual to find tiny strands of cytoplasm projecting from the periphery of the cytoplast toward and into the pit orifices (Fig. 76). The pits are long and narrow and apparently at times are branched (Figs. 77, 78).

Finally, about June 6th, the middle portion of the integument has become completely differentiated by the lignification process (Fig. 69). A longitudinal section of the nearly mature fruit collected on August 5th shows the thick stony layer to be a very rigid one of stone cells nearly uniform in thickness except at the mid-region of the seed where it is somewhat thicker and at the loci of the resin cavities where it is a little thinner than elsewhere. This lignification of the middle tissue of the integument brings about a striking change in the orientation of the axes of the cells comprising the integument, as pointed out by Quisumbing (52). Before lignification sets in, practically all the integumentary cells except the epidermis and the outer "fleshy" layer, which are elongated parallel to the surface of the developing seed, are nearly isodiametric and quite similar in general appearance. But very soon after the beginning of the lignification process the lignified cells become elongated perpendicular to the surface of the seed, and this manner of orientation persists to maturity. During this development, the cells of the inner papery layer become more numerous and those lying adjacent to the stony layer become somewhat pushed inward and crushed by the stony layer, thus forming a distinct line of demarcation, when viewed under high magnification (Figs. 71a, 72, 73, 80). A radial section about half-way between the top of the mature seed and its equator shows the integument to consist of the inner papery layer eight cells in thickness, the stony layer of about eight cell-layers and the outer "fleshy" layer of only one cell-layer plus the epidermal layer (Fig. 80). A detailed study of the stony tissue of the mature seed coat shows the inner- and outermost layers of these cells to be largely isodiametric and the intervening transversely elongated cells somewhat

tapered at their ends. Fig. 79 is a diagram of a longitudinal section of these three integumentary layers in their final proportions.

X. THE MEGASPORANGIUM

The Megaspore Mother-cell.—The development of the megaspore mother-cell, the megasporangium and the megaspores in *J. virginiana* is in accord with the descriptions by Norén (47) and Nichols (45) for *J. communis*. The ovulate flower-buds are formed in the axils of the leaves of only the young branches of the current season. They first appear in the latter part of the growing season prior to pollination. Although these buds are not recognizable to the naked eye until about a week before pollination, viz. about Feb. 15, dissected lateral buds on branches produced the season prior to pollination show young ovules already developed (Fig. 50b). At first, the integument extends high above the small nucellus; but the nucellar cells divide rapidly to form long parallel rows, and finally considerable differentiation takes place at the base of these long rows of nucellar cells to form a spherical mass of centrally placed cells at the base of the nucellus. There are at least 10–12 of these cells, and they constitute the female archesporium (Fig. 81). These archesporial cells and their nuclei are a little larger than the other nucellar cells, and they possess a denser cytoplast and 1–2 well-defined nuceoli (Fig. 81). The nucellar (vegetative) cells surrounding the archesporium become somewhat flattened and form more or less concentric layers. In *J. virginiana*, the archesporium is formed early in March before the formation of the cellular megagametophyte about May 25. This is at variance with the condition in *J. communis* which produces its archesporium about 12 months prior to megagametophyte formation and passes its winter after pollination in the archesporial stage (Nichols, 45). The ovules of *Pinus Strobus*, etc. (Ferguson, 25) and *P. palustris* (Mathews, 40) pass their first winter after pollination in an early free-nucleate (usually 32) megagametophyte stage.

Development of the Megaspores.—As Nichols (45) pointed out, it is impossible to predict with certainty which cells of the archesporial tissue will become the megaspore mother-cell, but it is usually one of the most centrally placed cells. In *J. virginiana*, the megaspore mother-cell becomes differentiated about March 20 (Fig. 82b) and just after germination of the pollen grains at the nucellar tip (Fig. 82a). This cell is considerably larger than the other archesporial cells. Its cytoplasm is rather dense and contains the conspicuous kinoplasmic body just below

the nucleus as described by Norén (47) and Nichols (45) for *J. communis* and for *Taxodium*, *Thuja*, and *Taxus* by Coker (16, 17), and *Torreya* by Robertson (53). Norén and Nichols have also shown that this body persists through the tetrad divisions of the mother-cell, and Nichols also reported that similar bodies are evident in the later stages of prothallial development. The other archesporial cells occupy positions surrounding the megaspore mother-cell and soon begin to form the tapetal (spongy) tissue.

No special effort was made in the present study to obtain all the stages in the tetrad divisions which produce the megaspores. Norén (47) made a detailed study of this development in *J. communis* and Nichols (45) added confirmatory information to Norén's account. These authors show that in *J. communis* the first division of the mother-cell nucleus gives rise to two nuclei bearing the haploid number of chromosomes. A permanent membrane is rarely, if ever, laid down between these nuclei. The first division thus resembles the first (heterotypic) division of the microspore mother-cell. Usually, only the lower of these two nuclei reaches entirely the resting stage. In this case, only the lower one undergoes the homotypic division, producing a group of three cells, only the lower two of which are true megaspores. Nichols (45) also found certain cases in which the spindles of this homotypic division in *J. communis* appeared similar to and were oriented similarly to the homotypic division in the microspore mother-cell of that species. In *J. virginiana*, only megaspore groups of three cells were ever found (Figs. 83–87), and only in rare instances were these cells seen to form a straight row. Even the middle megaspore of the group in Fig. 85 was somewhat out of line with the top and bottom megaspores. The tetrad divisions were completed by March 26th. Sludsky (62) has reported the presence of two megagametophytes in a single nucellus of *J. communis*, and Nichols (45) found as many as three megaspore mother-cells in tetrad division stages in the same species. No cases like these were observed in *J. virginiana*.

Immediately following the homotypic division, the functional megaspore, which is usually the lowest cell, or at least one of the lower cells, enlarges until it fills the entire space originally occupied by the mother-cell. Meanwhile, the nuclei of the non-functional cells disorganize rapidly (Figs. 86, 87), and are eventually absorbed. A unique condition for conifers in this respect was reported by Lawson (38) for *Pherosphaera*. In this Taxacean genus this author found that three megaspores are formed in an axial row and that all three germinate. The

upper one (viz. the one directed toward the micropylar end) undergoes three nuclear divisions but enlarges very little; the middle one advances to a later free-nucleate stage; while the basal megaspore enlarges more rapidly than the others and ultimately becomes the only one to produce a cellular female gametophyte.

XI. THE FEMALE GAMETOPHYTE

Development of the prothallium.—No special effort was made to obtain the details of the prothallial development in *J. virginiana.* Norén (47) made a thorough study of this process for *J. communis,* and *J. virginiana* agrees in all essentials of this development with Norén's description. Norén showed that the megaspore rapidly gives rise to an embryo sac containing a large central vacuole and a parietal layer of cytoplasm in which are embedded many free nuclei. Nuclear division takes place simultaneously throughout the sac. In the development of the prothallium, open tubes are formed which grow inward toward the center of the embryo sac in the manner first described by Mlle. Sokolowa (63) in 1890. The nucleus of each tube then divides, and cross walls are laid down. The continuation of this process ultimately gives rise to the cellular gametophyte. Nichol's (45) work on *J. communis* was essentially confirmatory of that of Norén in this phase of the life history. In 1900, Arnoldi (3) made a detailed study of the female gametophyte of *Sequoia sempervirens.*

The functional megaspore of *J. virginiana* gives rise to the 2-nucleate female gametophyte early in April. According to Nichols (45), the two nuclei produced by the division of the primary megaspore nucleus in *J. communis* var. *depressa* occupy a central position, and the cell contains several vacuoles. Nichols also observed in the same species that at the 4-nucleate stage these small vacuoles flow together to form one large central vacuole, and the cytoplasm with its included nuclei comes to lie about the periphery of the young embryo sac. By April 15, the young gametophyte of *J. virginiana* has reached its 8-nucleate stage (Fig. 88). Figure 88 shows the young gametophyte to be invested by a layer of loosely associated tapetal cells and a few of the adjacent nucellar cells. The tapetal cells persist in having denser cytoplasts than the nucellar cells until the gametophyte has become cellular. Later stages in the development of the gametophyte of *J. virginiana* are represented in Figs. 89 and 94. As pointed out by Norén (47) for *J. communis,* all the nuclei of the free-nucleate gametophyte of *J. virginiana* divide simultaneously. Figs. 90 and 91 show two stages in

the mitosis of two of these nuclei. Fig. 94 shows an intermediate stage in cell wall formation in the gametophyte.

Nichols (45) states that the volumetric ratio of the gametophyte of *J. communis* just prior to the formation of cellular tissue as compared with the megaspore at the time of its first division is about 12,500:1, and that the embryo sac finally assumes the shape of a prolate spheroid whose longitudinal axis measures about 1400μ. Miss Ferguson (25) found that the full-sized free-nucleate gametophyte of *Pinus Strobus* contains about 2,000 nuclei. These facts cited by Nichols and Miss Ferguson seem to agree quite closely with my observations on *J. virginiana*. Figs. 92 and 93 are diagrams of the nucellus and gametophyte of *J. virginiana* drawn to the same scale so as to show the comparative sizes of the 8-nucleate stage and the full-sized gametophyte just prior to its cell wall formation.

Mlle. Sokolowa (63) and Norén (47) state that no cross walls are formed in the early formation of the cellular gametophyte of *Juniperus* until the tube-like cells meet at the center of the prothallial cavity. Miss Ferguson (25) and Mathews (40) have shown that in *Pinus* cross walls are formed in these cells rather early in the growth of the prothallium, and Nichols (45) shows the latter to be true of *J. communis*. It is undoubtedly true of *J. virginiana* as well (Fig. 94).

Thomson (70) described the megaspore membrane of *J. sabina* and *J. virginiana*, and Norén (47) and Nichols (45) studied the megaspore membrane in *J. communis*. These authors agree that this coat is formed during the free nuclear period of the embryo sac and reaches its highest development at about the time of fertilization. It is about 3μ thick and about uniform in thickness except where it thins out considerably in the archegonial region of the prothallium. The coat is composed of two distinct layers, the fibrillar exosporium and the thicker homogeneous endosporium. Thomson stated that the exosporium is suberized, while the endosporium is largely cellulose. He also showed that the megaspore coat of gymnosperms is strikingly similar to that of the microspore coat.

The tapetum of the female gametophyte of gymnosperms is generally recognized to be homologous with the tapetum of the microsporangium. As Norén (47) and Nichols (45) have shown for *J. communis*, the tapetum of the microsporangium of *J. virginiana* as well as that of the female gametophyte is derived from the non-functional cells of the male and female archesporia, respectively. As the free-nucleate megagametophyte develops, these cells multiply rapidly and continue to invest the

young gametophyte. As stated above, these cells contain a rich supply of cytoplasm and numerous small starch grains. Nichols showed this layer to be still present when the cellular tissue of the gametophyte is being organized (Fig. 94), but subsequently rapidly disorganized.

The archegonium.—Between four and ten cells at the center of the extreme micropylar end of the female gametophyte are from the time of their formation a little larger than the other gametophyte cells and are destined to become the archegonia. Fig. 95 shows the very early differentiation of the archegonia on May 28, approximately one week before fertilization. One of the archegonial initials has already divided to cut off the neck initial cell above from the central cell below, while the other one is undergoing this division. As pointed out by Norén (47), the central cell nucleus is at first very similar in size and appearance to the nuclei of the surrounding gametophyte cells. Again, agreeing with *J. communis*, the group of 4–10 archegonia of *J. virginiana* forms a simple complex with no gametophyte cells between them. Figs. 67, 69, and 101 represent longitudinal sections of three different archegonial complexes. Interesting exceptions from this type of archegonial arrangement have been reported in closely related conifer groups. Lawson (35) shows that the numerous archegonia in the gametophyte of *Sequoia sempervirens* are variously grouped but always oriented with their necks directed to the side of the gametophyte, and he states that the archegonial initials arise from certain cells deep within the gametophyte tissue, rather than peripheral cells. Saxton (58) also reported lateral archegonia in *Actinostrobus pyramidalis*. In *Cunninghamia sinensis*, Miyake (42) showed that the archegonial complex usually has a sterile core of prothallial tissue at the center and that the tapetal or sheath layer of cells invests the outer margin of the archegonial complex.

The archegonia mature rapidly, and as is typical for the conifers bearing archegonial complexes, all the activities in all the archegonia of a complex usually occur about simultaneously. After cutting off the neck initial, the archegonium elongates considerably, and the neck initial cell divides twice to give rise to a tier of four neck cells (Figs. 96, 97). The young elongated archegonium contains a thin, peripheral layer of cytoplasm lining the archegonial wall which encloses a large central vacuole extending nearly the full length of the archegonium. The cytoplasmic layer is thicker at the upper end of the archegonium, and the central cell nucleus is embedded in this upper cytoplasm (Fig. 96).

By the time the archegonia have reached their full length the adjacent gametophyte cells have become organized into a single-layered jacket

or sheath completely investing the archegonial complex excepting the archegonial necks. These cells contain denser cytoplasts than their sister gametophyte cells, but like these gametophyte cells they are usually binucleate (Figs. 102, 111). Norén (47) and Nichols (45) stated that while the nuclear division which gives rise to this binucleate condition may be amitotic, mitotic figures have also been observed in the late history of the jacket cells.

Considerable attention has been paid to the peculiar granular deposits which appear in the archegonial cytoplast shortly before division of the central cell. These deposits together with the numerous cytoplasmic striations which radiate about them present striking aster-like configurations which are certainly a conspicuous feature of the archegonium of *J. virginiana*. Norén (47) and Nichols (45) have assigned to these structures the terms "Strahlungscentren" and "asteroids," respectively. Nichols stated that in *J. communis* "one of these asteroids is invariably situated in close proximity to the nucleus [central cell], and a second one may frequently be seen directly below this, while one or more are present in the lower part of the cell." The same arrangement seems to hold also in *J. virginiana*, but except for the constancy of the presence of one of these bodies just below the central cell nucleus, other asteroids are variously placed in different archegonia, and there may be several of these structures in a single archegonium. Several investigators have reported these structures in different groups of gymnosperms. Coker (16) discovered and described them thoroughly in *Taxodium* and suggested that their presence in the lower part of the long archegonium of *Taxodium*, which, as in *Juniperus*, has a large central vacuole separating the lower portion of the archegonium from the upper portion, may serve to provide "a more definite mechanism for the regulation of the entrance of the plastic material (nutrition) at this end." The asteroid located just below the central cell nucleus has been thought to function in the mitosis of this nucleus. (See Figs. 98, 99, 101.) As a rule, these bodies begin to disappear very rapidly soon after the mitosis of the central cell.

The proteid vacuoles, so common in the developing egg cytoplasm of gymnosperms, were first described by Hofmeister (28). They are rather numerous in *J. virginiana* (Figs. 100, 102, 103). They begin to appear at about the time of the central cell mitosis and to disappear soon after fertilization, but they are never as conspicuous nor as large in *J. virginiana* as in the Abietineae. These proteid vacuoles are generally agreed to be in some way concerned in the nutrition of the egg.

A thorough review of the literature on this subject has been presented by Stopes and Fujii (64).

Division of the Central Cell Nucleus.—Norén (47) and Nichols (45) gave thorough descriptions and demonstrations of the division of the central cell nucleus of *J. communis*, which produces the ventral canal nucleus at the top of the central cell cytoplast and the egg nucleus just below it.

The central cell nucleus of *J. virginiana* divides just two or three days prior to fertilization. All of the cells of an archegonial complex divide approximately simultaneously and very rapidly so that it is very difficult to obtain a complete series of stages of this division. The ventral canal nucleus also disappears very soon after it is formed. During this division, the archegonial cytoplast still contains a fairly large central vacuole. Just prior to the division, this nucleus shows a delicate reticulum with a conspicuous central nucleolus. The nuclear membrane on the side toward the asteroid becomes somewhat wrinkled and as described by Nichols, "pressed or drawn inward" (Fig. 98). Fig. 99 shows the telophase of this division. The chromosomes appear as delicate fibrous bands at the poles of the spindle. In Fig. 100 the division has been completed. The degenerate ventral canal nucleus is located at the top of the egg cytoplast, and the egg nucleus has become organized and considerably enlarged. Several investigators have believed that the spindle fibers for the central cell mitosis arise at the locus of the asteroid below the nucleus, but Nichols (45) was convinced that the asteroid does not contribute to the spindle formation "and its only apparent use in *Juniperus* is to form a support for the free lower pole of the spindle, . . ."

By the time of completion of the central cell mitosis, the central vacuole of the archegonium has vanished, the asteroids are disappearing, and the egg cytoplast begins to become rich in nutritive materials. The archegonial complex is surrounded by a specialized layer of jacket cells which are tapetal in nature (Figs. 101, 102). The eggs in the center of a complex are not, however, in contact with the jacket layer except at their lower ends. Since the cytoplasmic content of the centrally situated egg cells appears very similar to that of the outer eggs, it would seem, as Lawson (37) has suggested for *Libocedrus*, as if the food substances are translocated from egg cell to egg cell in the same way they are transferred from jacket cells to egg cell. Unlike the thick egg membranes of the cycads (Chamberlain, 13) and the Abietineae (Ferguson, 25; Mathews, 40), this membrane of *J. virginiana* is exceedingly

thin at the bottom but somewhat thicker toward the top of the archegonium (Figs. 102, 103).

XII. FERTILIZATION

After the division of the central cell nucleus, the lower daughter nucleus becomes the egg nucleus and rapidly enlarges and moves downward in the egg cytoplasm to a point just above the central vacuole. As noted above, however, very soon after this division, the central vacuole begins to diminish gradually until it soon vanishes entirely from the egg cytoplast. Meanwhile, the rapid acquisition of nutritive materials, accompanied by the formation of numerous proteid vacuoles, causes the egg cytoplasm to become much denser (Fig. 100). The young egg nucleus is ovoid and contains a close reticulum of linin threads on which are arranged irregular deposits of chromatin (Fig. 100). At the time of fertilization the egg nucleus is approximately 60 microns long and 42 microns in diameter.

Fertilization occurs about June 4th at Duke University, Durham, North Carolina. Fertilization stages were found in material from the same tree 2 to 3 days before and 2 to 4 days after June 4th. To sum up a few dates of important stages prior to fertilization: (1) the relatively rapid advance of the pollen tube downward from the upper third of the nucellar tip begins about 6 to 7 days before fertilization; and (2) the tip of the pollen tube enters the archegonial chamber above the archegonial complex about 1 to 3 days later; (3) the division of the body cell occurs about 2 to 4 days after the pollen tube has entered the archegonial chamber; (4) fertilization occurs about 2 to 4 days after the production of two sperm cells by division of the body cell.

Very thorough accounts of the fertilization in *J. communis* have been presented by Norén (46, 47) and Nichols (45), and the descriptions of these authors agree with the facts in *J. virginiana*. Figures 102 and 103 show two stages in fertilization in *J. virginiana*. The egg nucleus at the time of fertilization contains an irregular arrangement of linin threads on which are located very irregularly shaped chromatin deposits. Numerous pseudonucleoli (Norén, 47) are also present in the nucleus. Nichols (45) has shown for *J. communis* that just prior to fertilization the pollen tube membrane is dissolved or ruptured directly above the neck of an archegonium, and one of the male cells is squeezed through the neck to the egg, carrying the broken-down neck cells with it. There is no receptive vacuole in the egg of the investigated species of *Juniperus* as in the Abietineae. Nichols observed that the mem-

brane of the sperm of *J. communis* var. *depressa* is usually cast off before the sperm enters the egg, and he frequently found the remnants of the neck cells and the stalk and pollen tube nuclei of this species lying in the upper part of the egg cytoplasm immediately after fertilization. Unlike the condition in the Abietineae, where only the sperm nucleus farthest in advance in the pollen tube functions in fertilization so that only one egg is fertilized by one pollen tube, in *Juniperus* usually each of the two equal sperm cells effects fertilization in two separate archegonia. The relative sizes of the sperm nucleus of *J. virginiana* compared with that of the egg are about 1:4 as pointed out by Nichols for *J. communis* and not 1:1 as reported by Norén for the same species.

The male nucleus is accompanied or followed by its mantle of cytoplasm and starch as it rapidly approaches and finally comes in contact with the upper side of the egg nucleus (Figs. 102, 103). Coker (16) seems to have been the first investigator especially to emphasize the significance of the great abundance of starch furnished by the male cell in fertilization in a gymnosperm, viz. *Taxodium*. All students of gymnosperms prior to Coker's work seemed to agree that the male nucleus slips from its protoplast as it approaches the egg nucleus and leaves the protoplast behind near the point of entrance. Norén and Nichols described the entrance of the male protoplast to the egg in *Juniperus*. But until the present writing only the male nucleus in angiosperms as well as in other plants and animals has been considered as significant if even functional as a part in the fertilization phenomenon. Coker (15) says for *Taxodium* that "taking the occurrence of starch as an indication of the presence of leucoplasts, we find that most of the plastids of the proembryo of *Taxodium* are furnished by the male cell," since all signs of starch in the egg cytoplasm were negligible. This is certainly true for *J. virginiana*. However, as Coker pointed out, chromatophores or leucoplasts, or *mitochondria*, which require special methods to demonstrate, may well be present in the egg cytoplasm and therefore sooner or later contribute to the plastid contents of the embryo cells, although the quantity of starch granules supplied by the sperm cytoplasm compared to quantity appearing in the proembryonic cells seems to be approximately equal. In the light of a recent paper by Anderson (2) on *Antirrhinum majus* L., etc., who thinks it very likely that cytoplasmic inclusions (including plastids) are transmitted from the pollen tube to the egg during fertilization to combine with similar-appearing inclusions already surrounding the egg nucleus, it might be that male starch and plastids are brought into association with female

inclusions (mitochondria, etc.) during fertilization in *J. virginiana* so that the ultimate embryonic cells may be supplied with a male and a female complement of cytoplasm and cytoplasmic inclusions. As far as the visible starch content of the proembryonic cells of *J. virginiana* is concerned, however, at least its plastids seem to have been entirely furnished by the male cell (Figs. 102-109; 114), and with each mitosis of the proembryo after fertilization, each daughter cell receives a supply of the starch present in the zygote cytoplasm. Norén (47) noted an increase in the starch content in the zygote cytoplasm after fertilization in *J. communis*. There is also an increase in the volumetric proportion of starch at this time in *J. virginiana* compared with that of the starch sheath of the sperm before fertilization. The obvious explanation of this is that each small granule (plastid?) brought in by the sperm cytoplast receives an additional deposit of starch after it enters the egg cytoplast, causing a marked increase in the total volume of starch.

As may be noted in Figs. 102 and 103, a conspicuous vacuolation is left in the egg cytoplasm behind the sperm as it advances to unite with the egg nucleus. This has already been explained by Norén (47) as being due to the sperm's pushing the egg cytoplasm downward and sidewise out of its path. The sperm nucleus usually causes a considerable depression in the egg nucleus (Fig. 103), as has been described for other conifers, and comes to lie in this depression. The sperm nucleus reaches the egg nucleus just above the center of the egg and immediately thereafter both of the nuclei move to the base of the egg cytoplast where they become invested by the starch sheath derived from the sperm cell and their fusion ultimately occurs. Nichols (45) concluded that in *J. communis* dissolution of the sperm and egg nuclear membranes occurs at their points of contact before any indication of the first segmentation division appears.

XIII. EMBRYO FORMATION

Development of the proembryo.—Blackman (5), Chamberlain (12), and many other investigators of conifers have shown that the chromatin substance of the male and female nuclei may be distinguished in the early preparation of the fusion nucleus to divide. Blackman also pointed out that it is only when the "half chromosomes derived from the male and female nuclei respectively fuse together at the poles of the first segmentation spindle" that fertilization may be considered as completed. Norén (47) states that in *J. communis* the chromatin of the male and female nuclei is gradually transformed into pseudonucleoli

which become grouped on either side of the dividing membranes of the fusion (zygote) nucleus. These pseudonucleoli then become arranged into two spiremes and the mitosis proceeds to completion. Nichols (45) describes for *J. communis* var. *depressa* that the zygote nucleus rests for one or two days after fusion and following this there gradually begins to be constructed in the nucleus what appears to be two spiremes of moniliform threads well differentiated into chromatin and linin. These spiremes then draw themselves together and the segmentation division occurs. Fig. 104 shows the metaphase of the first division of the fusion nucleus in *J. virginiana*, and Figs. 105a and 105b represent the resting stage of the first two sporophytic nuclei produced in this division. The chromosomes of the early sporophytic mitoses are strikingly slender curved rods (Figs. 104, 106, 110). The diameter of the fusion nucleus at this time is exceptionally great, and the faint protoplasmic meshwork described by Nichols (45) as being sometimes noticeable in the mature egg nucleus before fertilization is striking at this time (Fig. 104). The fusion nucleus together with the investing starch sheath almost completely fills the base of the archegonial cavity from side to side.

The poles of the mitotic spindle of the fusion nucleus are so arranged that the two resulting groups of chromosomes become located one above the other in the base of the archegonium. After the completion of the first segmentation division, and during the organization of the two resting nuclei, the investing layer of cytoplasm and starch presses in between the two daughter nuclei so that each nucleus becomes surrounded by approximately an equal amount of starch (Figs. 105a, 105b). Thus a 2-nucleate proembryo, or the first two nuclei of the sporophyte, is formed.

The subsequent divisions which give rise to the 12-celled proembryo occur in rapid succession, three days being the maximal time required. The second sporophytic division (Fig. 106) gives rise to 4 free nuclei which arrange themselves in a tetrad at the base of the archegonial cytoplast, each nucleus still being well invested by the starch accumulation (Fig. 107). The third sporophytic division produces eight free nuclei which become variously arranged at the base of the archegonial cytoplasm (Figs. 108, 109); but the usual arrangement seems to be that of three tiers, in which the order from top to bottom is 4, 3, 1 nuclei as in Fig. 109. Wall formation now sets in to cut off each of the eight sporophytic nuclei together with a quantity of cytoplasm and starch, leaving only the four cells of the upper tier open to the archegonial

cytoplasm above. The cytoplasm of these early sporophytic cells shows a marked radiation about the nuclei with cytoplasmic strands extending to and connecting with the limiting plasma membranes (Fig. 109).

Soon after the 8-celled proembryo stage, the four cells of the upper tier divide simultaneously (Fig. 110) to form the 12-celled proembryo, of which only the four cells of the uppermost tier remain open above. These are termed the rosette tier of the proembryo (Fig. 111). This marks the end of the proembryonic development that takes place prior to the elongation of the proembryo suspensors. The proembryonic development up to this point in *Juniperus* agrees with the other Cupressineae and *Pinus* (Ferguson, 25; Mathews, 40) and the other Abietineae. But as shown by Coulter and Chamberlain (19) and Mathews, a further division occurs in the lowest tier of the proembryo of *Pinus* prior to elongation.

Miss Ferguson (25) showed that in *Pinus* all the divisions which occur prior to the establishment of the 12-celled proembryo take place "under the direct influence of the egg-cytoplasm . . . suggesting . . . a closer relationship with those lower gymnosperms in which many free nuclei arise in the egg before the deposition of cell-walls." Immediately following the production of the 12-celled proembryo in *J. virginiana*, the tier of cells just below the open rosette tier begins rapid elongation (Fig. 111). At this time the formerly active jacket cells of the archegonial complex appear considerably elongated, degenerated and crushed. Active tapetal function is now assumed by the gametophyte cells just below the archegonial complex. Iodine stained preparations reveal these cells to contain numerous starch grains, and their cytoplasts are dense and apparently very active. Gradually a sort of "cord" or "core" of such cells becomes distinguishable in a progressive development from below the archegonial complex and extending down the center of the gametophyte.

Origin and Formation of the Embryo.—Hofmeister (28) made a few observations on the embryogeny of the Cupressineae in his investigations of *Thuja occidentalis*, *Juniperus communis* and *J. virginiana*. Land (32) included a treatment of this subject in his morphological study of *Thuja*; Saxton made a series of studies of the embryogeny of *Tetraclinis* (59), *Widdringtonia* (55, 56), *Callitris* (57) and *Actinostrobus* (58); and more recently Buchholz has made detailed examinations of this phase of development of *Biota* (7) and *Chamaecyparis* (9). Saxton found the embryogeny as well as other phases of the life histories of

Tetraclinis, *Widdringtonia*, *Callitris* and *Actinostrobus* so distinctive and different from other investigated Cupressineae that he suggested placing these four genera in a separate tribe of conifers, the Callitrineae, coordinate with the Cupressineae. Hence, except for Land's study of *Thuja* in 1902, embryological investigations of species which are unquestionably Cupressinean had been comparatively meagre until Buchholz's critical examination of *Biota*, etc. (7), in 1926 and *Chamaecyparis obtusa* (9) in 1932. Using a combination of paraffin serial sections and Buchholz's dissection technique (Buchholz, 7), the following is the first detailed account to appear on the later embryogeny of a species of *Juniperus*.*

In the conifer proembryo corresponding with that of Fig. 111 of this paper, the group of cells below the elongating so-called suspensor tier of cells seems to be generally regarded as "embryo initial" cells. The subsequent history of these cells does not bear out this idea, unless an "embryo initial" cell may be so flexible a term as to embrace a cell which can give rise to a suspensor initial cell and an embryonic cell. Buchholz showed for *Biota* (7) and *Chamaecyparis* (9) that during the elongation of the first suspensor cells to elongate, which he terms "prosuspensors," and the pushing of these "embryo initials [end cells of proembryo] into the enlarging cavity of the gametophyte," the embryo initials divide at their tips to produce a large cell above and small cell, the embryonic cell, at the end. Then this author states that "almost immediately the larger proximal cells so cut off form suspensors which correspond to the primary suspensor cells in *Pinus*."

In the early proembryo of *Juniperus virginiana*, it is clear that all the eight cells below the rosette tier are elongating cells, and that embryo initials which give rise by mitoses directly to embryo cells, do not exist in the 12-celled proembryo (Figs. 111–117). The first cells of the proembryo to elongate are those of the prosuspensor tier just below the rosette tier, and considerable elongation occurs the next day after the stage shown in Fig. 110 (Fig. 111). As the prosuspensor cells elongate, the bulk of the cytoplasm of each cell becomes collected at the lower ends (i.e. the morphological apical end of the proembryo) of the cell and contains the nucleus while only a slight parietal layer is

*While this article was in galley proof a paper on "A new type of embryogeny in the conifers" by Miss Phyllis L. Cook appeared in the March number of the American Journal of Botany. In this paper she showed that in *Juniperus communis* the peculiar development of the embryo was essentially the same as brought out in the present paper.

left at the top (Fig. 111). Apparently as soon as the proembryo has filled the archegonial cavity and some pressure is exerted at the ends, the cells at the morphological apex ("S" Fig. 112) begin to elongate, and very soon these last cells to elongate become so oriented that the distal ends of the prosuspensor cells become more or less serially adjoined to the proximal ends of the elongating end cells (Figs. 113, 115). The combination of a prosuspensor cell and an elongating end cell may be regarded as a *suspensor unit* even until after the embryo is produced at the anterior end.

As both tiers of suspensor cells continue to elongate, the tips of those of the lower tier present a decidedly haustorial appearance and contain numerous starch grains embedded in the dense cytoplasm at the tip (Fig. 114). These tips push between the gametophyte cells, gradually digesting them and preparing a rather spacious cavity in the gametophyte for the proembryo (Fig. 115). Soon the whole group of suspensor units begins to coil in the same general direction, and meanwhile the suspensor cells may divide one or more times (Figs. 116–119a). Many cells usually become detached from the surrounding gametophytic tissue and adhere to the suspensor cells where they are gradually absorbed.

After the suspensor units have advanced a little more than one-fourth of their final lengths (Figs. 116, 117), the distal cell of each suspensor unit undergoes mitosis (Fig. 118a, 118b), giving rise to the true embryo initial at the tip and a suspensor cell immediately above (the primary suspensor of Buchholz, 7). Figs. 118a, 118b were made from a paraffin microtome section. Figs. 119a and 119b show the whole proembryo and an enlargement of the tip of a suspensor unit (respectively) just after this mitosis. As may be noted from the above figures and subsequent ones, cleavage polyembryony exists in *J. virginiana* as Buchholz has shown for *Biota* and *Chamaecyparis*. After their establishment, the embryo initials remain dormant for a period of 3 to 4 days while the suspensor cells are rapidly elongating. In addition to the further elongation of the suspensor, the further development of the embryo system entails rapid mitoses of the embryo initial cells, and the establishment of a secondary suspensor by at least one of the suspensor units. Many whole proembryos at different stages of development were carefully dissected to determine the number of cells of which the suspensor portion of the single units is composed. Four such units are shown in Figs. 120a–120d. It is clear that in *J. virginiana* (1) the entire suspensor just prior to the formation of the secondary suspensor is composed of 4–7 cells; (2) the cells of the suspensor constitute a

definite unit and are therefore not as loosely associated as Buchholz has shown for *Biota* and *Chamaecyparis*; and (3) there is some evidence of the proliferation of secondary embryos from prosuspensor cells (Figs. 117, 120a, 120d, 129, 130), but this is not as pronounced as Buchholz found in *Biota* and *Chamaecyparis*.

By the time the terminal primary embryo has reached the 8-celled stage (Fig. 122a), the proembryo system has reached about its maximal length, measuring up to $2\frac{3}{4}$ mm. (in the coiled state), and the entire system has become a much coiled structure, frequently having as many as 8 turns in it.

As in other conifers that show cleavage polyembryony, the primary embryo (terminal) which becomes and remains most advanced in its development becomes also the final mature embryo of the seed in *J. virginiana*, while the remaining primary embryos as well as any secondary embryos that may be proliferated from prosuspensor cells become abortive and are eventually absorbed. Strasburger (63) studied the development of the primary embryo from the embryonic initial cell in *Thuja occidentalis* and *Juniperus* and found the development rather similar. The essentials of this development in *J. virginiana* are in accord with Strasburger's description (his p. 307). Although the early primary embryonic cell-groups (viz. before secondary suspensor formation occurs) may frequently be formed in various ways (Figs. 120d, 123, 124, 125), usually the embryonic initial cell (Fig. 119b, etc.) divides by a transverse wall which establishes two tiers in the embryonic group (Fig. 121). The lower (end) cell gives rise to only embryonic cells, while at least some of the cells produced by mitoses of the upper member of this pair give rise to the secondary suspensor to be described later. Each of these cells now undergoes numerous mitoses in rapid succession, the first mitosis in the upper cell being longitudinal but transverse in the lower cell. Thus a more or less two-tiered embryonic group of about 12 cells is formed as shown in Fig. 123. The primary suspensor cell bearing this group has become very large, often 55 microns in diameter, and contains a large nucleus near its lower end. All the suspensor cells now contain a scanty supply of cytoplasm.

Buchholz states that the early multicellular embryos of *Biota* (7) and *Chamaecyparis* (9) seem to develop at least for a brief period by apical cell growth as was also noted by Strasburger (66) for *Thuja occidentalis*. I was unable to detect this method of development in the embryo of *J. virginiana*. There were, however, some indications

of fragmentation of the young multicellular embryos of *J. virginiana* (Figs. 120d, 126a), thereby resulting in a secondary cleavage stage in the embryo, as Buchholz found to be a not uncommon characteristic in *Biota*; but *J. virginiana* agrees more with *Chamaecyparis* (Buchholz, 9) in that such a condition is very rare.

By the time the embryonic cell mass has reached a stage of approximately 125–150 cells, the upper (proximal) cells of the cell group begin to elongate posteriorly from the embryo and apply themselves closely to the primary suspensor in the manner described by Buchholz (9) for *Chamaecyparis obtusa*. (See my Figs. 126a, 126b.) These cells continue to elongate and to divide until they produce the massive secondary suspensor system of Buchholz (7, 9). (My Figs. 127–130.) In the backward growth of the secondary suspensor system, the old primary suspensor is observed to become detached and gradually displaced by these elongating suspensors (Figs. 127, 128). Meanwhile the entire primary suspensor system, including all primary embryos except the anterior functional one, begins to collapse and degenerate and will finally be entirely absorbed by the time the functional embryo is mature (Figs. 128–130).

While the secondary suspensor system is developing, the mass of polyhedral embryonic cells at the distal end of the embryo system undergoes rapid mitoses, and throughout at least the majority of the embryonic development, all the observable secondary suspensor units are seen to be in series with the early established vertical rows of embryonic cells below (Figs. 128, 129).

For a considerable period there is no indication of organal differentiation of the embryo, but by July 20 and before the embryo is half grown, the dome-shaped meristematic bud of the stem tip (plumule) becomes recognizable (Fig. 129). The two broad cotyledonary primordia appear about the same time as the plumule. Buchholz (6) showed that the stem tip meristem of *Pinus* is visible prior to the appearance of the cotyledonary primordia, but the reverse is true in *Sciadopitys* (Buchholz, 8) in which species this author stated that "the stem tip is probably delayed until after the seed begins to germinate." The plerome apex of the root in the embryo of *J. virginiana* is recognizable before the plumule and cotyledonary primordia, as Buchholz has also shown for *Pinus* and *Sciadopitys*. The embryo of *J. virginiana* reaches maturity by the last of July, a little over seven weeks after fertilization (Fig. 130). The principal change that occurs from the first appearance of the plumule and cotyledonary primordia until

maturity is a general elongation of the cotyledons and hypocotyl, since the embryo has attained approximately its maximal diameter by the time these organs first appear. A gradual reduction and absorption of the old primary suspensor system has taken place by this time so that very little, if any, of it exists, although a rather elaborate secondary suspensor still remains attached to the upper margin of the root cap. The root cap agrees with *Sciadopitys* in which, according to Buchholz (8), the root-cap embraces less than one-fourth of the embryo, and not with *Pinus*, whose root-cap, according to the same author (6), includes nearly half of the embryonic axis. In the median longitudinal section of the mature embryo of *J. virginiana* (Fig. 130), the approximate arrangement of the cell layers is shown. There are about seven distinct longitudinal layers from the median stele structure to the margin of the embryo, and about 17 layers comprise the dome-shaped root-cap. Fig. 79 shows the proper proportions and relationships of all the tissues which surround the embryo in the mature berry-cone at this time.

XIV. EMBRYOGENY OF THE CUPRESSINEAE AND RELATED GROUPS

In reviewing the investigations of the embryogeny of the Cupressineae and closely related groups, there seem to be at least two types known for the Cupressineae, exclusive of those four genera which have been considered as comprising the Callitrineae (see p. 43 above). Strasburger (66), Land (32) and Buchholz (9) have described the production of only a single embryo from the fertilized egg of *Thuja* (simple polyembryony). On the other hand, cleavage polyembryony, or the production of several embryos by a single fertilized egg, has been reported for *Biota* (Buchholz, 7), *Chamaecyparis* (Buchholz, 9), *Libocedrus* (Lawson, 37), and *Juniperus* (see present paper). It should be noted here that several investigators of the Taxodineae (notably Coker (16) on *Taxodium*, Lawson (36) and Buchholz (10) on *Crytomeria japonica*, and Buchholz (8) on *Sciadopitys*) have reported cleavage polyembryony in this subfamily and proembryos similar to those of the Cupressineae. However, Buchholz (9) points out in his paper on *Chamaecyparis* that "it appears that the Taxodineae usually have no primary suspensor" but "have prosuspensors which are morphologically equivalent to the structures so-named in *Chamaecyparis*, on the ends of which the embryo initials develop into multicellular embryos without the intervention of the very long primary suspensor." Saxton's investigations of the embryogeny of

Tetraclinis, *Widdringtonia*, *Callitris*, and *Actinostrobus* (see above), have shown that the archegonia of these genera occupy a lateral position in the female gametophyte and that wall formation in the proembryo occurs at an earlier stage (i.e., as early as during the transition from the binucleate to the four-nucleate condition in *Actinostrobus* and *Callitris*). Saxton reports that the proembryo completely fills the archegonium in these genera, and he shows diagrammatically for *Actinostrobus* that just prior to cutting off "embryo initials" the proembryo is composed of six cells, an apical tier of two cells separated by a wall longitudinal to the long axis of the proembryo and four cells beneath this tier separated by transverse walls. The suspensors elongate laterally from the proembryo but toward the base of the gametophyte, since the proembryo itself is laterally located in the gametophyte. The zygotes all have cleavage polyembryony as is true of the other investigated Cupressineae except *Thuja*.

XV. SUMMARY

Juniperus virginiana L. is a dioecious conifer native to eastern United States and southern Ontario. This paper is a study of the morphological and cytological development of the sporophylls and seed of this species.

The staminate cones begin to develop in August and attain their mature size by winter. Three to four microsporangia develop as marginal evaginations at the base and on the lower side of the microsporophyll; and immediately distal to the microsporangia, the downward development of a broad, flattened outgrowth from the microsporophyll completes the formation of the sporophyll's peltate shape. The large resin cavity of the microsporophyll originates schizogenously.

By late September the pollen grains are formed and contain an abundance of large starch grains. By the time the exine of the mature pollen grain is complete the following spring, this starch supply has disappeared. The mature sporangial wall consists of a single layer of secondarily thickened cells.

Meiosis of the pollen mother-cells resembles that of other conifers. The mother-cell nucleus is about one-third as large as that of *Larix* and *Pinus*, and the reduced number of chromosomes is eleven. At meiosis, the mother-cell cytoplasm presents a strikingly alveolar appearance due to its starch content, and at completion of the first

meiotic division, starch grains show an alternate arrangement on either side of the protoplasmic layer midway between the two resting daughter nuclei.

No further division occurs in the pollen grain until after pollination.

No sterile prothallial cells are formed, and the mature pollen grain is wingless.

Pollination occurs between the middle and last of February. The internal cellulose bands of the microsporangial wall cells facilitate sporangial rupture in a manner comparable to the thickened portions of the annular cells of leptosporangiate ferns. The pollination-drop is particularly noticeable in *J. virginiana* at the time of pollination.

The division giving rise to the generative cell and the tube cell resembles that of *Taxodium* but occurs after pollination. The pollen grain germinates soon afterwards; the generative cell divides to form the body cell and stalk cell nucleus about three weeks later, by which time the pollen tube has grown some distance at the tip of the nucellus.

As in other conifers, the early growth of the pollen tube is slow and considerably branched in the nucellar tip. About the last of May, however, it shows a much more active and direct growth, and within a very few days penetrates to the necks of the archegonia.

The mature body cell possesses a dense cytoplast which contains a rich starch supply as is characteristic of Cupressineae, except possibly *Widdringtonia* (Saxton, 56). This cell divides about three days before fertilization (i.e., about June 1st) and simultaneously with the central cells of the archegonial complex. The resulting two male cells are equal and have contents and a thick membrane similar to the body cell.

The ovulate cones begin their development about the first of September and continue slight growth during the winter. They are borne terminally on axillary branches of the current season. Usually two pairs of megasporophylls, pairs *a* and *b* from summit of cone-axis downward, are borne decussately on the short cone-axis, but sometimes a lower pair or a rather rudimentary apical pair may enter into cone formation. Only sporophyll pair *b* is fertile. In approximately two-thirds of the female flowers, only one sporophyll is fertile and bears a single ovule, and in about half of the remaining flowers the one fertile member of pair *b* bears two ovules, while both members of pair *b* of the remaining flowers are fertile, each bearing one ovule. The ovules appear about the middle of September and are borne in the

axil of the fertile megasporophylls. An outgrowth, the fruit-scale, develops on the morphological upper side of the megasporophylls. Like the more distally located cover-scale of the sporophyll, the fruit-scale soon becomes supplied with a separate vascular bundle. The fruit-scales of the four megasporophylls continue to grow upward and finally coalesce above the ovule or ovules to produce approximately the upper half of the outer fleshy coat of the small, blue berry-like cone.

The mature berry-cone of *J. virginiana* has a stony integumentary layer which forms a protective coat entirely surrounding the seed. The secondary thickening of the walls of the cells which produce this layer begins developing about May 1st in the micropyle-closing layer and progresses downward through the middle region of the integument until the completion of the lignification process about June 6th. The thick-walled stone cells of this layer bear for the most part long and narrow simple pits.

While the ovules appear about the middle of September, the megasporophylls are not recognizable to the naked eye until about the time of pollination the following spring. During pollination, the single megaspore mother-cell may be distinguished near the base of the nucellus. Reduction division gives rise to two nuclei, only the lower of which divides equationally. The three resulting cells do not usually lie in a straight row but are more frequently grouped in a manner resembling the tetrad arrangement of the microspores. One of the two lower megaspores resulting from the second division develops into the female gametophyte, the other two cells disorganizing.

The archegonia, usually four to ten in number, are arranged in a close complex at the center of the extreme micropylar end of the female gametophyte as in other Cupressineae. The complex is surrounded closely by a tapetal layer which is more densely supplied with cytoplasm than its surrounding sister megagametophytic cells. Each archegonium has a tier of four neck cells. The central cell is long and contains several "asteroids" in its cytoplasm. An extremely short-lived ventral canal nucleus is cut off rapidly and simultaneously in all the central cells of the archeogonial complex about three days before fertilization.

Fertilization occurs from about June 1st to 7th at Chapel Hill, N. C. Usually the two equal male cells of a pollen tube effect fertilization in two archegonia. The male nucleus is somewhat smaller than the

egg nucleus. As in other Cupressineae, as well as in Taxodineae, the starch-filled male cytoplast accompanies the nucleus in fertilization, passes through the cytoplasm at the tip of the egg and during fusion of the two sexual nuclei entirely enfolds them. This starch and cytoplasm also accompany the fusion nucleus when it subsequently passes to the base of the archegonium and are more or less equally distributed to the cells of the proembryo.

Resulting from three divisions of the fusion nucleus, eight free nuclei are formed at the base of the archegonium and become variously arranged but usually so that one nucleus is lowermost. Cell walls are now formed but the upper tier of nuclei, usually four, is left open. This tier now divides by walls at right angles to the long axis of the archegonium, producing the rosette of free nuclei above and the so-called suspensor tier immediately below.

In the twelve-celled proembryo of *J. virginiana*, all the eight cells below the rosette tier elongate, the upper tier, however, elongating first. Cleavage polyembryony is assured early, since the tips of the morphologically anterior suspensor cells are separated early by their varied directions of penetration of the megagametophyte. Soon, however, a cavity is digested in the gametophyte allowing the suspensor units to become more closely associated. After the suspensor units of a whole proembryo have grown about a fourth of their final length (i.e., about .6 mm.) and each unit is composed of two to three suspensor cells, the anterior cell of each unit divides to produce an apical embryo initial cell and the primary suspensor cell. The whole suspensor portion of the proembryo continues to elongate in a spiral fashion, sometimes reaching the ultimate length of 2.75 mm. and showing seven to eight turns in its spiral structure. Meanwhile, the suspensor cells continue to divide, and each suspensor unit is often composed of four to seven cells.

By the continued division of the embryo initial cell at the tip of each suspensor unit, a small group of embryonic cells is formed by the time the suspensor units have completed their elongation. This embryonic group can sometimes be seen to be somewhat constricted into two tiers of cells. The upper cells of the upper tier soon begin to elongate posteriorly to produce a very extensive secondary suspensor system as in *Biota* and *Chamaecyparis*, while the anterior tier of cells contributes most to formation of the embryo proper. Thus the embryo initial of each suspensor unit of the proembryo gives rise to a separate embryo thereby producing several embryos from a

single archegonium. Usually the developing embryo which is most anterior becomes larger than the rest and produces the final single embryo of the seed.

The mature embryo is dicotyledonous and reaches maturity about the last of July.

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XVII. EXPLANATION OF PLATES

PLATE 1

Fig. 1. Longitudinal section of male branch and bud before male cone is recognizable. r.c., resin cavity. × 17. Aug. 8.
Fig. 2. Longitudinal section of male cone in earliest stages of microsporangial origins. × 17. Aug. 8.
Fig. 3. Longitudinal section of a nearly mature male cone. sg., sporangium. × 17. Sept. 23.
Fig. 4. Mature male cone. × 3.

Fig. 5. Longitudinal section of microsporophyll before sporangial formation sets in. ch.c., chlorophyll-bearing cells; r.c., resin cavity; r.ch., resin channel; r.cl., resin cell. × 110. Aug. 8.

Fig. 6. Longitudinal section of microsporophyll showing very early stage in sporangial formation. r., resin. A plate of three archesporial cells is present here. × 110. July 28.

Fig. 7. Later stage in sporangial formation. Arrow indicates mid-line of cone axis. × 110. July 28.

Fig. 8. Longitudinal section of portion of microsporophyll showing young sporangium with central primary sporogenous tissue surrounded by one-layered tapetum and the two-layered sporangial wall. r.c., resin cavity. × 110. July 28.

Fig. 9. Microsporangium with mature pollen mother-cells. p.m.c., pollen mother-cell; t., tapetum. × 110. Sept. 23.

Fig. 10. Fate of the enveloping walls of microsporangium. Inner sporangial wall layer degenerating. t., tapetum. × 490. Sept. 23.

Fig. 11. Microsporangial wall layers and tapetum after young pollen grains are developed. The pollen grains are richly supplied with starch. × 490. Sept. 23.

Fig. 12. Longitudinal section of mature microsporophyll before pollination. The outer sporangial wall layer alone persists. p.g., pollen grain; r.cl., resin cell. × 56. Feb. 12.

PLATE 2

Fig. 13. Microsporangial wall cells. Longitudinal view. n., nucleus. × 552. Feb. 12.

Fig. 14. Microsporangial wall cells. Cross section view; somewhat diagrammatic. × 552. Feb. 22.

FIG. 15. Fully mature pollen mother-cell. × 1200. Sept. 17.

Fig. 16 (1). Mother-cell nucleus approaching synizesis. Pairing of the chromatin material is taking place,—zygonema stage. × 1200. Aug. 21.

Fig. 16 (1)a. Portion of the chromatin structure of Fig. 16 (1). × 1200.

Fig. 16 (2). Synizesis. × 1200. Aug. 21.

Fig. 17. Nucleus recovering from synizesis. Open spireme beginning to form. × 1200. Aug. 21.

Fig. 18. Open spireme, uniformly distributed. Alveolar (or frothy) appearance of cytoplasm from here on is due largely to the accumulation of a rich starch content. × 1200. Aug. 21.

Fig. 19. Pachynema stage. Obviously paired chromatin material shows transverse segmentation. × 1700. Sept. 23.

Figs. 20, 21. Strepsinema stages. The chromatin segments are shortening, thickening and somewhat twisting. × 1200. Aug. 21.

Fig. 22. Near diakinesis stage. Chromosomes have attained compact form just prior to formation of the spindle. × 1200. Aug. 21.

Figs. 23, 24. Meta- and telophases of the heterotypic division. × 1200. Aug. 21.

Fig. 25. Resting daughter nuclei of the heterotypic division. The characteristic cytoplasmic configuration midway between the nuclei is due to thicker cytoplasm here and a sort of alternate arrangement of large starch grains on either side of it. × 1200. Sept. 23.

Fig. 26. Polar view of resting dyad nucleus. × 1200. Sept. 23.

Fig. 27. Telophase of homotypic division. × 1200. Sept. 23.

Fig. 28. Cell plates being formed between the cells of the young tetrad. The cytoplasm is rich in starch. × 1200. Sept. 23.

Figs. 29, 30. Polar views of stages as in Fig. 28, showing the haploid number of chromosomes to be eleven. Fig. 29, × 1700; Fig. 30, × 1200. Sept. 23.

Fig. 31. Young tetrad of microspores surrounded by pollen mother-cell wall. × 1200. Sept. 23.

Fig. 32. Microspores within thick-walled chambers of tetrad. × 1200. Sept. 23.

Fig. 33. Microspores being liberated from tetrad chambers. × 1200. Sept. 23.

Fig. 34. Young microspore after being freed from tetrad chamber. × 1200. Sept. 23.

Fig. 35. Intine and exine walls developing around pollen grain. × 1200.

Fig. 36. Mature pollen grain with cytoplasm rich in starch in resting condition prior to the winter rest period. × 1200. Sept. 23.

Fig. 37. Pollen grain just before pollination. Very little, if any, starch is now present. × 1200. Feb. 12.

Figs. 38–40. Pollen mother-cells and a microspore dyad showing reaction to starch test in different stages of development. × 1200. Aug. 21.

Fig. 41. Pollen grain prior to germination at tip of nucellus. nuc., nucellus. × 1200. Feb. 25.

Figs. 42, 43. Early germination of pollen grain at tip of nucellus. The mucilaginous pollination-drop is present at the tip of the nucellus at this time. g.c., generative cell; p.t.n., pollen tube nucleus; p.d., pollination-drop. × 1200. Mar. 22 and 26, resp.

PLATE 3

Fig. 44. Pollen tube penetrating tip of nucellus. p.t.n., pollen tube nucleus. Generative cell (g.c.) has changed from lenticular to spherical shape. × 490. Mar. 30.

Fig. 45. Pollen tube and contents after division of generative cell, the whole still near tip of nucellus. b.c., body cell; st.n., stalk cell nucleus; p.t.n., pollen tube nucleus. × 490. Apr. 19.

Fig. 46. Pollen tube and contents. The body cell is acquiring a densely granular cytoplast. b.c., body cell. × 490. May 19.

Figs. 47–49. About the time of fertilization. (See below.)

Fig. 47. Just prior to division of body cell (b.c.) and subsequent to division of central cell. The body cell cytoplast is filled with starch granules. The egg nucleus has not attained yet its full size. eg., egg cell. × 234. June 4.

Fig. 48. After division of body cell to form two equal sperm cells (sp.). × 234. June 3.

Fig. 49. Median section of a mature sperm cell and edge of its partner, the latter shown in outline. × 530. June 3.

Fig. 50a. Young female branch when cone branches are first recognizable. Branches marked "X" bear very young ovules at their tips. × 5. Sept. 16.

Fig. 50b. Ovule from a branch "X" of Fig. 50a. nuc., nucellus. × 56.

Fig. 51. Most common type of female flower, showing three opposing pairs of sporophylls and one ovule. From the tip of the floral branch, they run in order a, b, c. One member of pair "b" is fertile. The fertile sporophylls are underlined. × 8. About Mar. 29.

Fig. 52. An uncommon flower type with an additional pair of sporophylls, a′, which is sterile and above pair a on the cone axis. × 8. About Mar. 29.

Figs. 53a, 53b. A not uncommon flower type with one of the members of pair b being fertile and bearing two ovules. Contrary to the one-ovule type, in the two-ovule type, the sporophyll pair c usually enters into the sporphyll fusion to produce the berry-cone. × 8. About Mar. 29.

Fig. 54. Two-ovule flower type in which one ovule is borne by each member of sporophyll pair b. × 8. About Apr. 5.

Figs. 55–59. Female floral diagrams to show the most common floral configurations. The sporophyll pairs which usually enter into the berry-cone formation are shaded in, and the order from cone apex downward is labeled from a′ and a, etc. ov., ovule. × 24 and × 44.

PLATE 4

Fig. 60a. Mature one-seeded berry-cone in side view showing cover scale tips, the fertile member of pair b (underlined) being solitary. The seed is outlined. × about 4. Jan.

Figs. 60b, 60c. Views at right angle to that of Fig. 60a.

Fig. 61. Mature two-seeded berry-cone in side view. In this type, only one member of sporophyll pair b is bearing the two seeds. × about 2. Jan.

Fig. 62. Mature two-seeded berry-cone in side view, in which each member of sporophyll pair b is bearing one seed. × about 2. Jan.

Fig. 63. Longitudinal section view of female flower just after pollination. Indication of the sporophyll-outgrowth (ovuliferous- or fruit-scale) may be noted here. p.g., pollen grain; m.m.c., megaspore-mother-cell. × 17. Mar. 5.

Fig. 64. Later stage of cone development. The sporophyll outgrowths are advancing above the necks of the ovules, and their separate vascular supplies may be noted. o.s., fruit- (ovuliferous) scale; c.s., cover-scale. × 17. Mar. 30.

Fig. 65. After fusion of the sporophyll-outgrowths above necks of ovules. × 17. Apr. 15.

Fig. 66. Female cone at the beginning of secondary thickening of the integument to form the stony layer of the seed. s.t., stony tissue; m-g., megagametophyte. × 17. May 9.

Fig. 67. Berry-cone showing further progress in stony tissue formation. p.t., pollen tube; s.t., stony tissue; nuc., nucellus; ar., archegonium; m-g., megagametophyte. × 17. May 26.

Fig. 68. Longitudinal section showing method of closure of sporophyll-outgrowths at tip of berry-cone. cu., cuticle. × 110. About May 26.

Fig. 69. Seed in longitudinal section view just after fertilization to show diagrammatically the nearly final stage in the stony layer development. × 17. June 6.

Fig. 70. Tip of integument in longitudinal section view to show details of beginning of stony tissue formation. Cell "X" is about the first cell below the micropyle-closing cells (mi. cl.) to show the secondary thickening. × 234. May 1.

Fig. 71a. Slightly later stage in stony tissue formation. The outer "fleshy" layer (o.f.), one cell in thickness and just beneath the epidermis, and the inner papery layer (i.p.) may now begin to be differentiated from the middle stony layer (s.t.). × 234. May 9.

Fig. 71b. Details of two cells from Fig. 71a. The normal and active protoplasts are shrunken due to fixation. × 530.

PLATE 5

Fig. 72. Further stage in secondary thickening of stone cells adjacent to inner papery tissue cells (i.p.). p., pit. × 530. May 26.

Fig. 73. Longitudinal section about mid-way the integument of an ovule of the stage of Fig. 67. o.f., outer "fleshy" layer; s.t., stony tissue; i.p., inner papery layer. × 234. May 26.

Fig. 74. Later stage in secondary thickening of stone cell. Surface view. × 523. June 4.

Fig. 75. Section views of stone cell wall in late stage of thickening. ×·1063. p., pit. June 4.

Fig. 76. Stone cells in longitudinal view showing pits and apparently normal and active protoplasts with projections from their periphery into the pit openings. s.w., stone-cell wall. × 490. June 4.

Figs. 77, 78. Transverse and longitudinal views of mature stone cells of the seed coat stained with safranine and Delafield's haematoxylin. The nuclei are still present. × 490. Aug. 5.

Fig. 79. Longitudinal section view of a mature berry-cone to show proportions and relationship of parts. spl., sporophyll; o.f., outer "fleshy" layer; s.t., stony tissue; i.p., inner papery layer; nuc., nucellus; m-g., megagametophyte; v.-o.s., vascular supply to fruit-scale; v.-c.s., vascular supply to cover-scale; e., embryo; r.c., resin cavity. × 18. Aug. 8.

Fig. 80. Histological details of the portion of Fig. 79 embraced by the two parallel lines and brace near top of figure. r.ca., resin channel. Remainder of abbreviations as in Fig. 79 above. × 56.

PLATE 6

Fig. 81. Group of sporogenous cells (about 8 in all) surrounded by spongy tissue (sp.t.) at base of nucellus. The larger upper cell would probably be the functional megaspore mother-cell. × 530. Mar. 5.

Fig. 82a. Tip of ovule after germination of pollen grain. p.g., pollen grain; mi., micropyle; p.d., pollination-drop; nuc., nucellus. × 163. Mar. 22.

Fig. 82b. Megaspore-mother-cell (m.m.c.) differentiated at base of nucellus in same ovule as in Fig. 82a. × 530.

Figs. 83–87. Triads of megaspores. The megaspores are not generally arranged in straight rows. In Fig. 85, the lowest megaspore is below the level of the other two. (See below.)

Fig. 83. Megaspore triad. × 530. Mar. 22.

Fig. 84. Megaspore triad. × 530. Mar. 26.

Figs. 85, 86. Megaspore triads. × 530. Mar. 24.

Fig. 87. Triad of megaspores. × 1063. Mar. 30.

Fig. 88. Early free-nucleate megagametophyte (n.m-g.) bearing 8 nuclei in all, surrounded by loosely associated spongy tissue (tapetum) and a few nucellar cells. × 234. Apr. 15.

Fig. 89. Later free-nucleate stage of megagametophyte. n.m-g., free-nucleate megagametophyte; t., tapetum; nuc., nucellus. × 234. Apr. 17.

Figs. 90, 91. Prophase and metaphase of division of nuclei in the parietal layer of cytoplasm of the young megagametophyte. × 530. About Apr. 17.

Figs. 92, 93. Comparative sizes of nucellus (nuc.) and free-nucleate megagametophyte (n.m-g.) at the earliest and latest development of the latter. × 16. Fig. 92, Apr. 15; Fig. 93, May 21.

Fig. 94. Proportions and relationship of megagametophytic (m-g.), tapetal (t) and nucellar (nuc.) layers at the time of early wall formation in the megagametophyte. × 234. May 21.

Fig. 95. Division of archegonial initials at summit of megagametophyte (m-g.) to produce the neck initial cell (n.i.) and central cell (c.c.). m.m., megaspore membrane. × 490. May 29.

Fig. 96. Tangential section of young archegonial complex showing an archegonium (ar.) surrounded by jacket layer (tapetum). n., dividing neck cell. × 234. May 26.

Fig. 97. Tier of archegonial neck cells in horizontal plane. × 490. May 29.

PLATE 7

Figs. 98–100. Division of central cell nucleus to cut off ventral canal nucleus (v.c.n.). This division occurs very rapidly, and the ventral canal nucleus disappears quickly after formation. The large central vacuole of the central cell rapidly vanishes while the cytoplasm becomes richer in food material. Meanwhile, the newly formed egg nucleus (eg. n.) enlarges considerably, and the nucleolymph stains light brown with haematoxylin and orange G. × 234. Fig. 98, June 3; Figs. 99, 100, June 2.

Fig. 101. Proportions and relationship of parts of ovule just before divisions of body cell (b.c.) of pollen tube (p.t.) and central cells (c.c.) of archegonia. o.f., outer "fleshy" layer; s.t., stony tissue; i.p., inner papery layer; nuc., nucellus; p.t.n., pollen tube nucleus; t., tapetum; m-g., megagametophyte. × 56. May 29.

Fig. 102. Portion of upper part of an archegonium and adjacent jacket layer (t.) and megagametophyte cells (m-g.) at time of fertilization. s.n., sperm nucleus; s.c., sperm cytoplasm; eg.n., egg nucleus. × 490. June 4.

Fig. 103. Basal part of archegonium soon after fertilization showing sperm nucleus (♂) with its enveloping starch-filled cytoplast in contact with the egg nucleus (♀). Haematoxylin preparation. ar.m., archegonial membrane; p.v., proteid vacuole. × 530. June 4.

Fig. 104. Division of the fusion nucleus (fertilized egg). s.s., starch sheath. × 530. June 6.

Fig. 105a. Archegonial complex showing a zygote with its two basal nuclei invested by the rich supply of starch, resulting from the first division of the fusion nucleus. × 57. June 5.

Fig. 105b. Details of the basal portion of the 2-nucleate zygote of Fig. 105a. × 530.

Figs. 106, 107. Details of the second division of the fusion nucleus and the resulting four daughter nuclei. × 490. Fig. 106, June 5; Fig. 107, June 7.

Figs. 108a, 108b. Archegonial complex (× 58) and details of basal portion of 8-celled proembryo (× 490). Cell wall formation has set in, each cell possessing a nucleus and cytoplast with a rich supply of starch. June 6.

Fig. 109. Eight-celled proembryo, the tier of 4 cells at the morphological base open above. × 490. June 4.

Fig. 110. Divisions (simultaneous) of the tier of 4 cells of the morphological base of the proembryo to produce the open rosette tier above and suspensor tier below. × 490. June 4.

PLATE 8

Fig. 111. Proembryo, with prosuspensors (four in one tier) beginning to elongate. Some of enveloping megagametophyte cells (m-g.) are shown. ps., prosuspensors; s., end cells of proembryo; r.t., rosette tier. × 234. June 7.

Fig. 112. Elongation of the suspensor cells (s.) at the morphological apex of the proembryo has begun. × 234. June 8.

Fig. 113. Later elongation of proembryo. × 110. June 4.

Fig. 114. Morphological apex of proembryo, showing penetration of the megagametophyte tissue by the suspensor units. s., end cells of proembryo. × 234. June 7.

Fig. 115. Later development of proembryo (from paraffin section). The megagametophyte cells adjacent to the tips of the suspensor units (s.) possess a denser cytoplast in which many starch grains are imbedded. m-g.c., sloughed off megagametophyte cells which have become attached to the suspensor cells; p.c., proembryonic cavity within megagametophyte. × 110. June 9.

Figs. 116, 117. Later suspensor units from two different ovules, dissected to show their cellular structure. "1" and "2" of Fig. 116 are obviously the end-cells (s.) of their particular suspensor units. In "3", a prosuspensor cell has just divided. Fig. 117 shows the beginning of proliferation of a secondary embryo (a.e.) from a suspensor cell. All suspensor cells have megagametophyte cells (m-g.c.) adhering tightly to their edges. These are apparently sloughed off mechanically during the elongation of the proembryo. The contents of these cells are used up *in situ*. × 110. June 11.

Fig. 118a. Later proembryo (from paraffin section), showing division of the end cell of a suspensor unit to cut off the embryo initial cell (e.d.) at the tip (leaving the primary suspensor cell immediately above). × 56. June 17.

Fig. 118b. Details of the mitosis of Fig. 118a. × 490.

Fig. 119a. Later proembryo after its suspensor units have cut off their embryo initials. pr.s., primary suspensor; e.i., embryo initial cell. About June 11.

Fig. 119b. Details of tip portion of a suspensor unit of Fig. 119a. (See Fig. 119a.) × 234.

PLATE 9

Figs. 120a–d. Dissected suspensor units at different stages of development. The prosuspensor of a unit, as a rule, seems to be comprised of more cells the nearer it approaches maturity. The proliferation of secondary (prosuspensor) embryos (a.e.) seems to be much less common in the longer proembryos than the shorter ones. pr.s., primary suspensor; e., primary embryo; m-g.c. megagametophytic cells. × 43.

Fig. 121. Daughter cells resulting from mitoses of the embryo initial cell. s.s.i., secondary suspensor initial cell. × 234. About June 16.

Fig. 122a. Later proembryo, entire, showing the characteristically coiled arrangement of the maturing proembryo. pr.s., primary suspensor; e., primary embryo. × 33. About June 18.

Fig. 122b. Details of the terminal embryonic group of cells of the proembryo of Fig. 122a. × 110.

Figs. 123–125. Later primary embryos and their primary suspensors above. That of Fig. 123 is a more typical case. Figs. 123, 125, × 234; Fig. 124, × 110. June 18-24.

Fig. 126a. Later development of a whole proembryo. Embryonal tubes (cells) are growing back from the morphological basal part of primary embryo to produce the secondary suspensor. × 33. About June 25.

Fig. 126b. Details of the terminal primary embryo (e.) of Fig. 126a. pr.s., primary suspensor; e.t., embryonal tubes (cells). × 110.

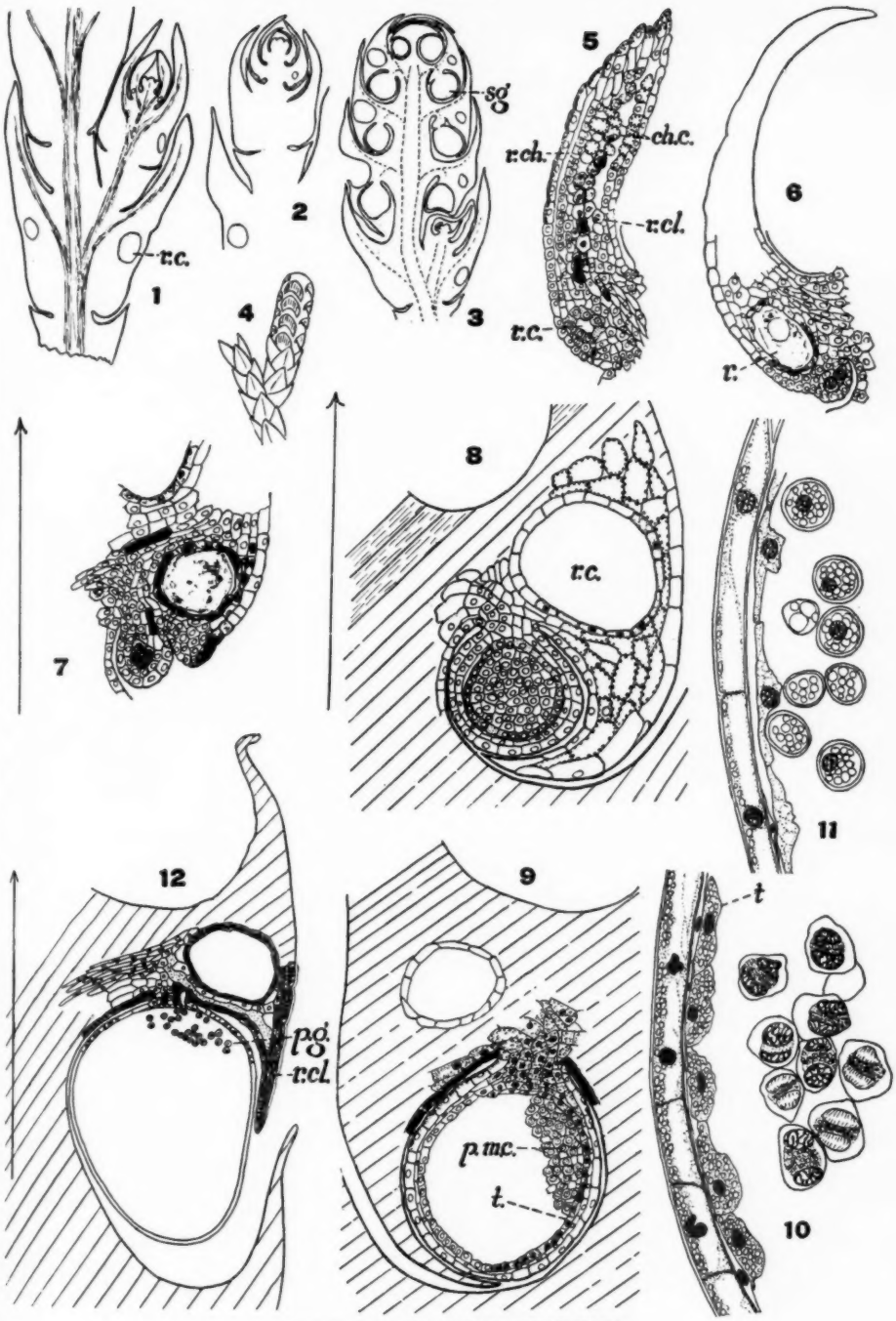
Fig. 127. Later primary embryo. The primary suspensor seems to be dislodged from its original attachment and pushed backward by the developing secondary suspensor cells. × 110. About June 25.

Fig. 128. Later proembryo, showing further development of the functional primary embryo (e.) and the secondary suspensor (s.ss.) above. pr.s., primary suspensor; ps., prosuspensor. × 33. About July 20.

Fig. 129. Early appearance of the plumule (plu.) and the two cotyledons (cot.). Many nuclei are observable in the old prosuspensor units above but are not shown here. × 33. About July 24.

Fig. 130. Mature primary embryo. The parallel lines indicate the arrangement of the peripheral cell layers. plu., plumule; cot., cotyledon. × 24. July 28.

PLATE 1



MATHEWS on JUNIPERUS VIRGINIANA

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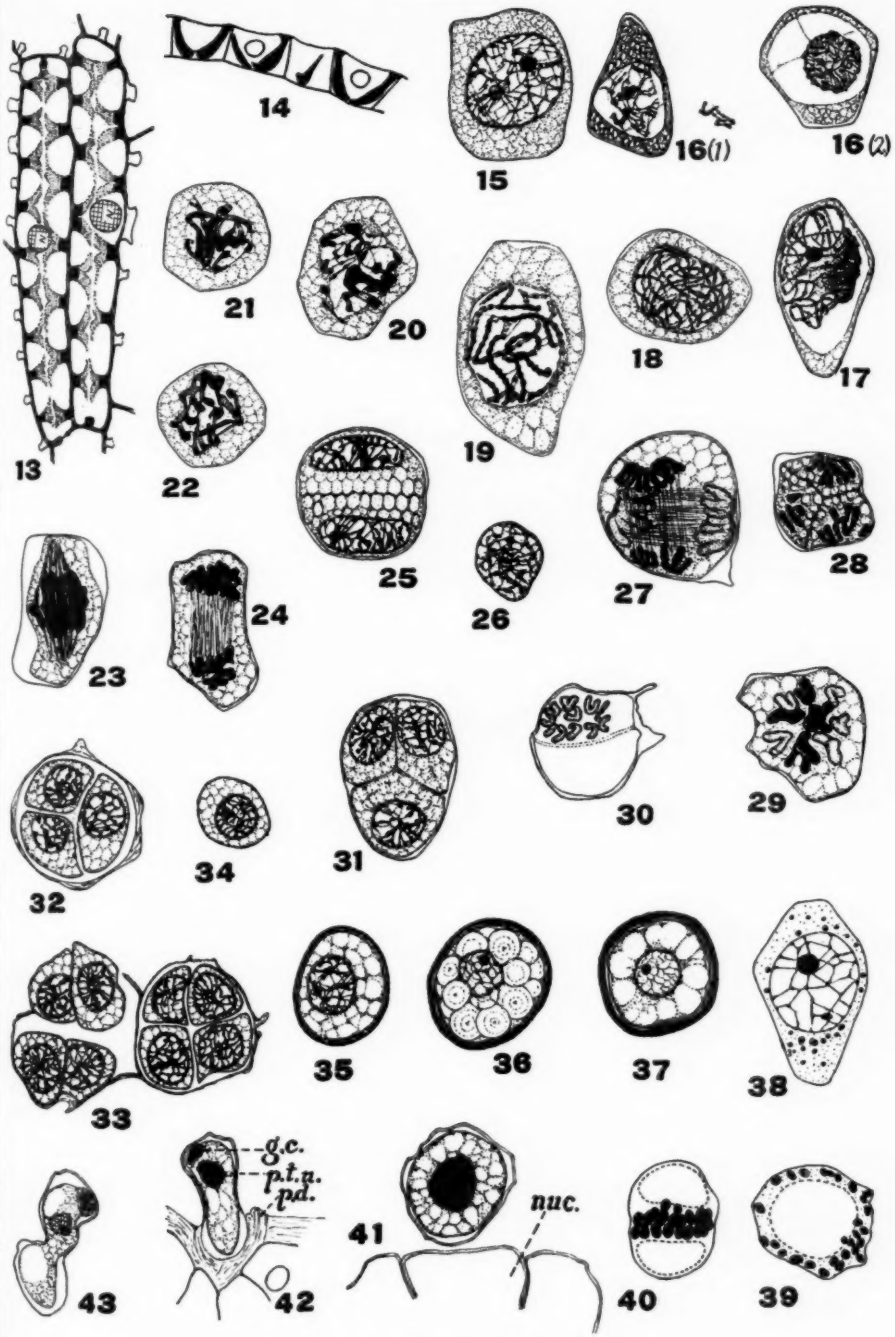
PLATE 2



MATHEWS on JUNIPERUS VIRGINIANA

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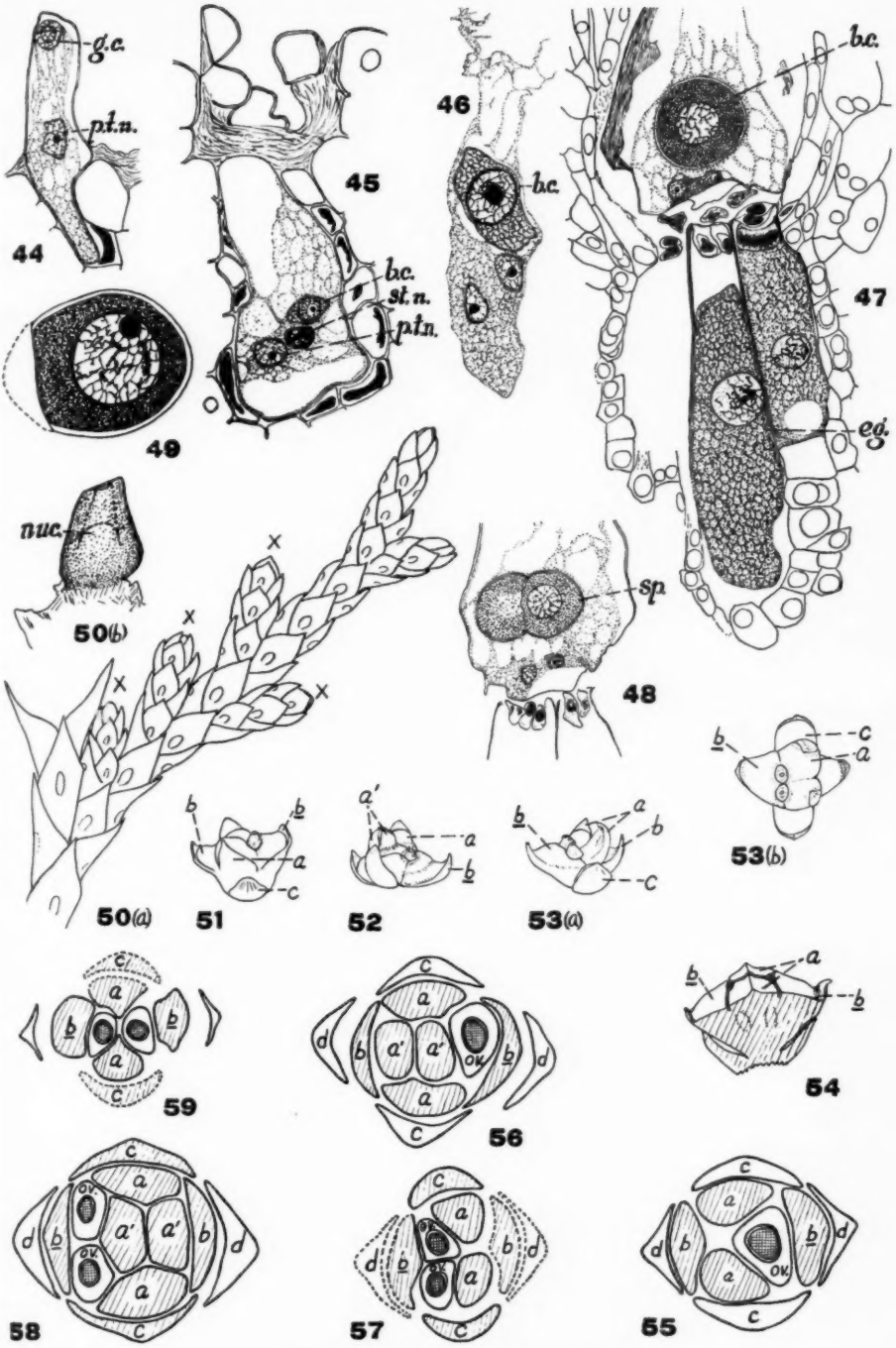

MATHEWS on JUNIPERUS VIRGINIANA

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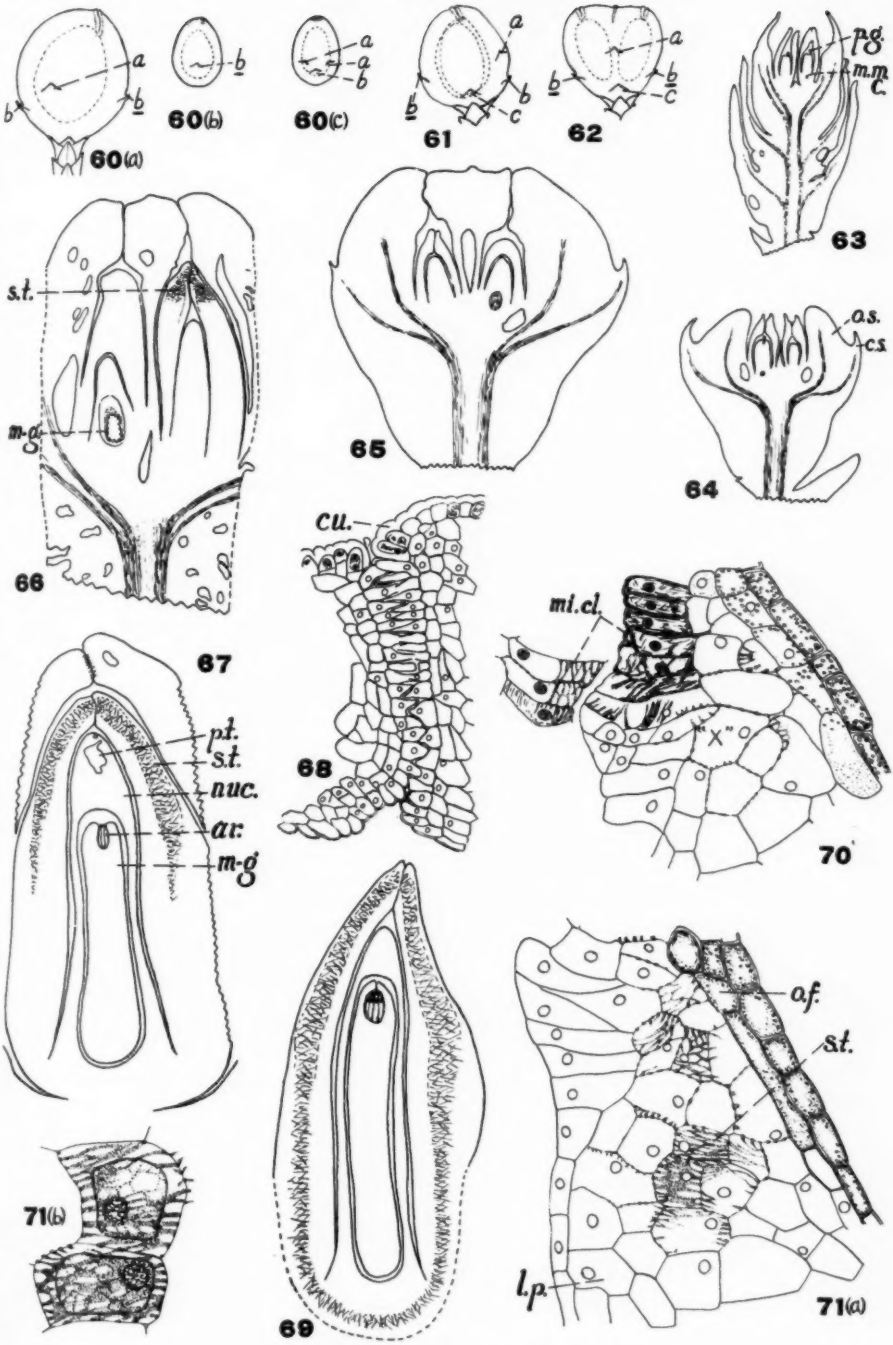
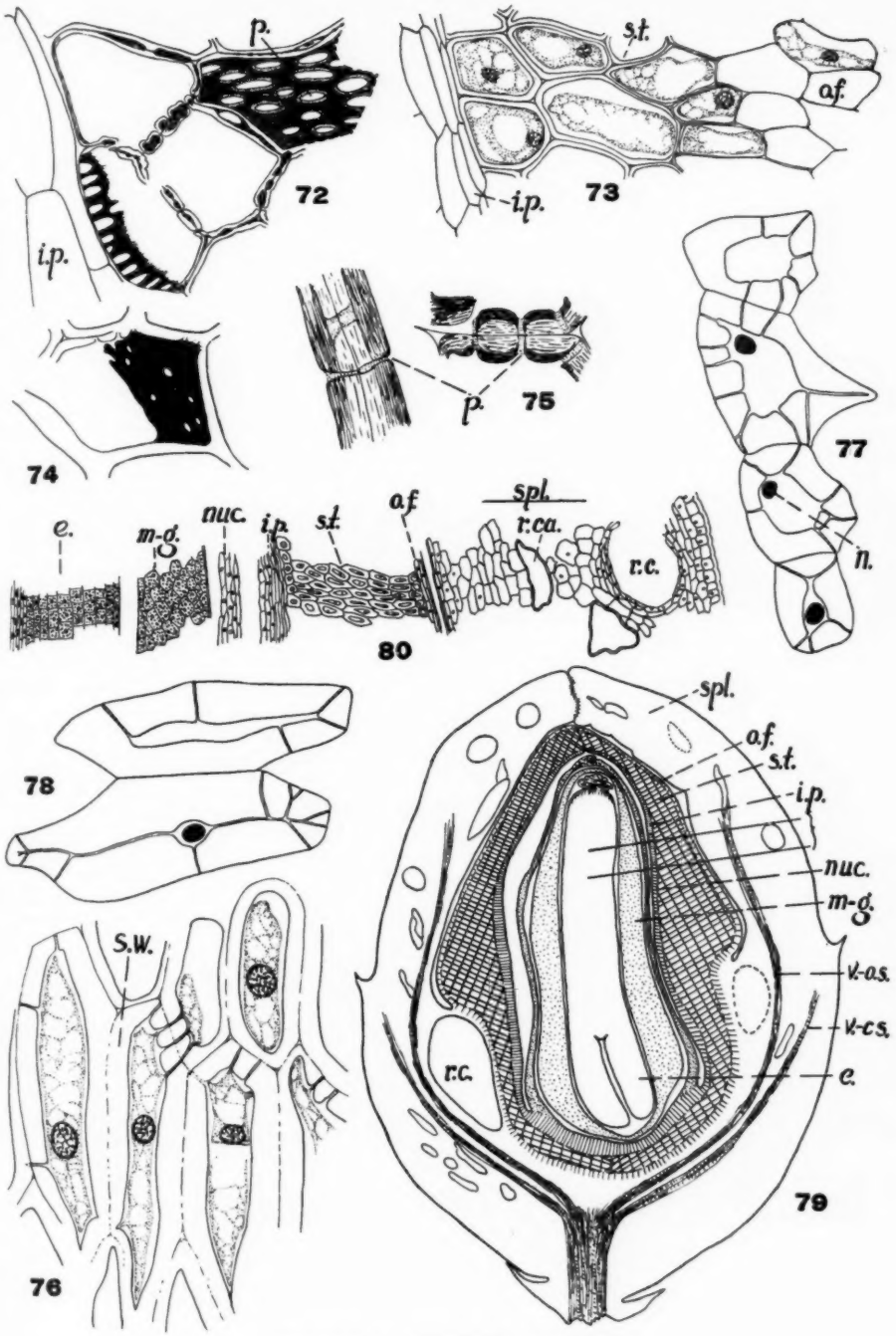
PLATE 4



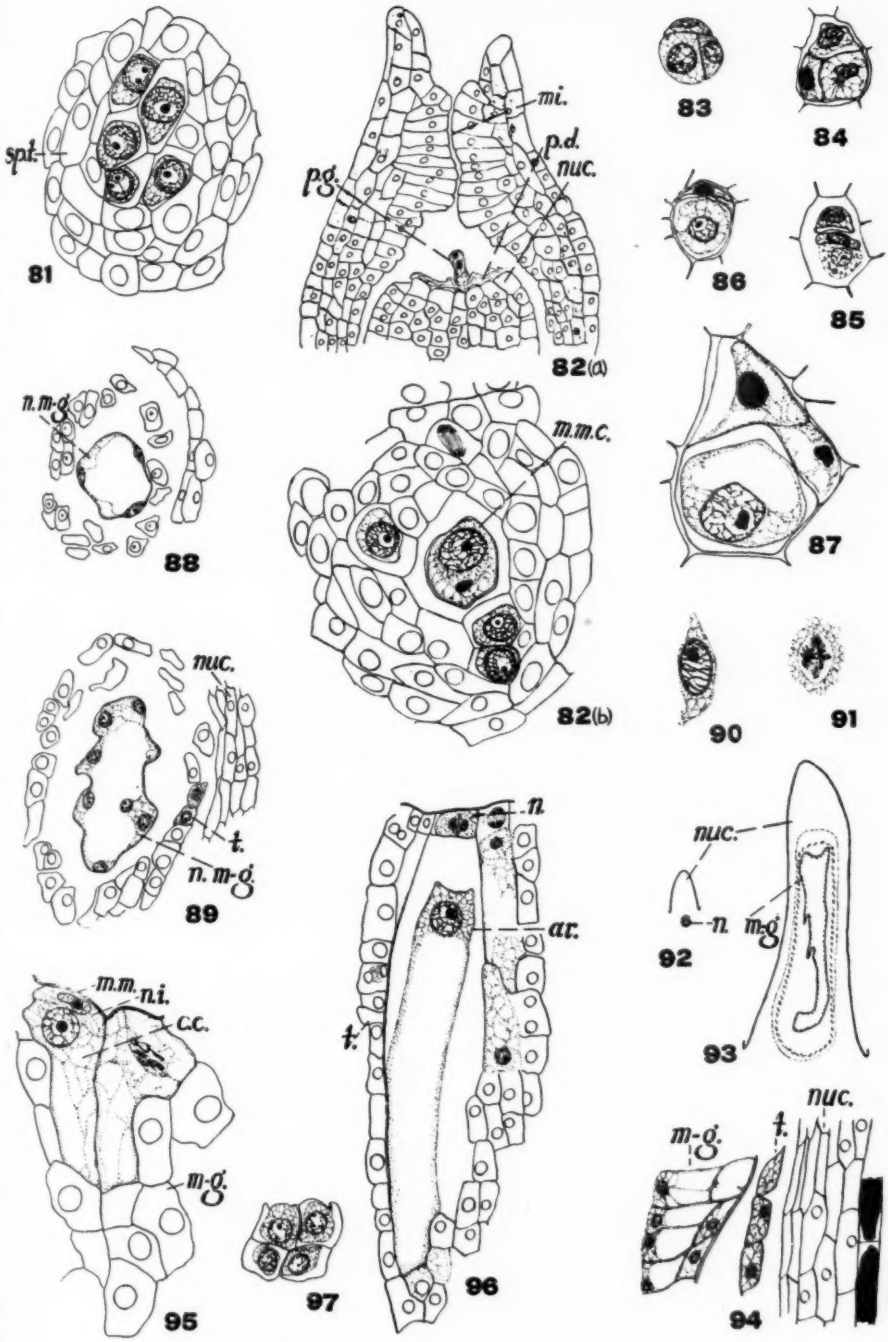
MATHEWS on JUNIPERUS VIRGINIANA

PLATE 5



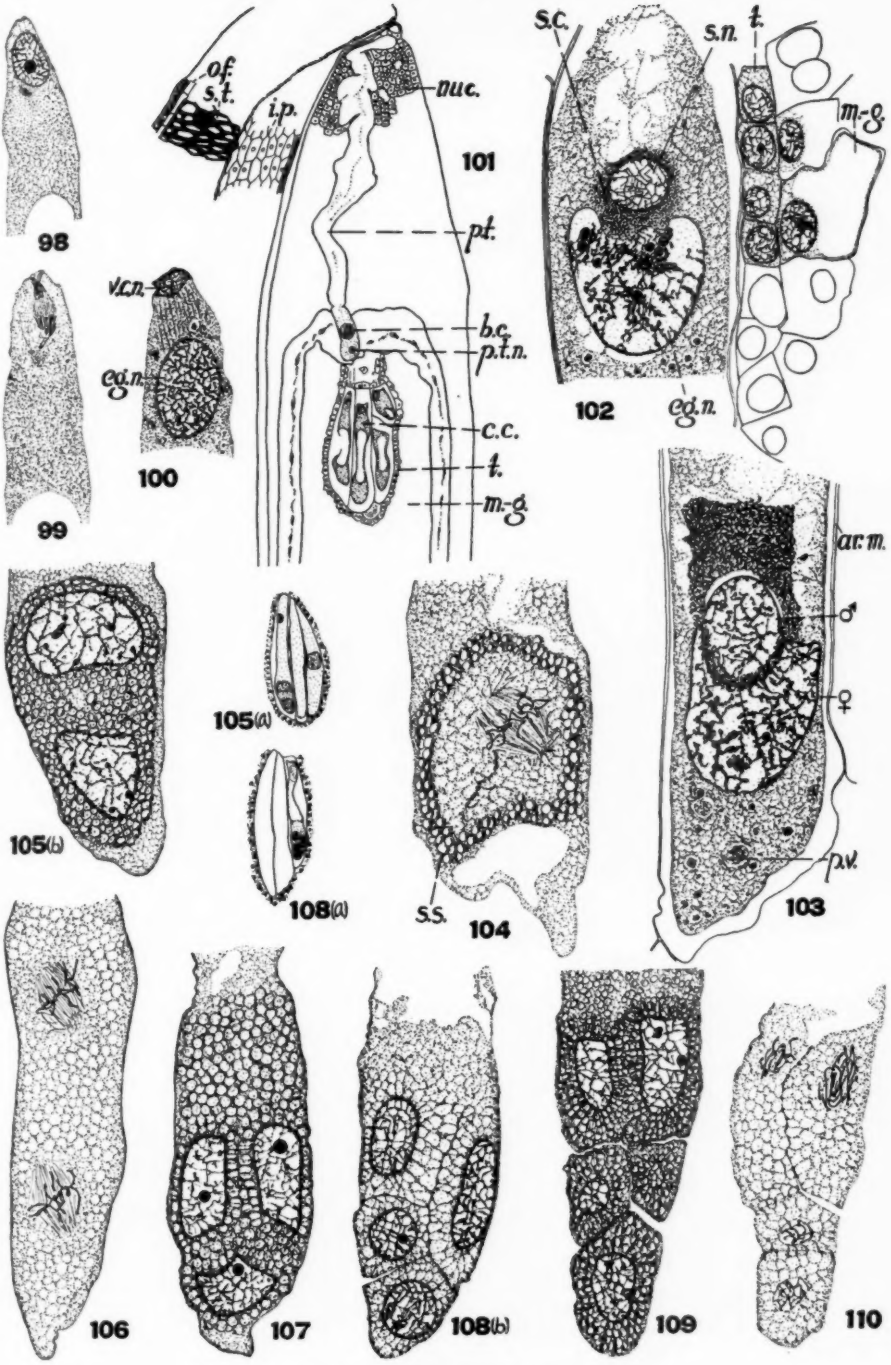
MATHEWS on JUNIPERUS VIRGINIANA

PLATE 6

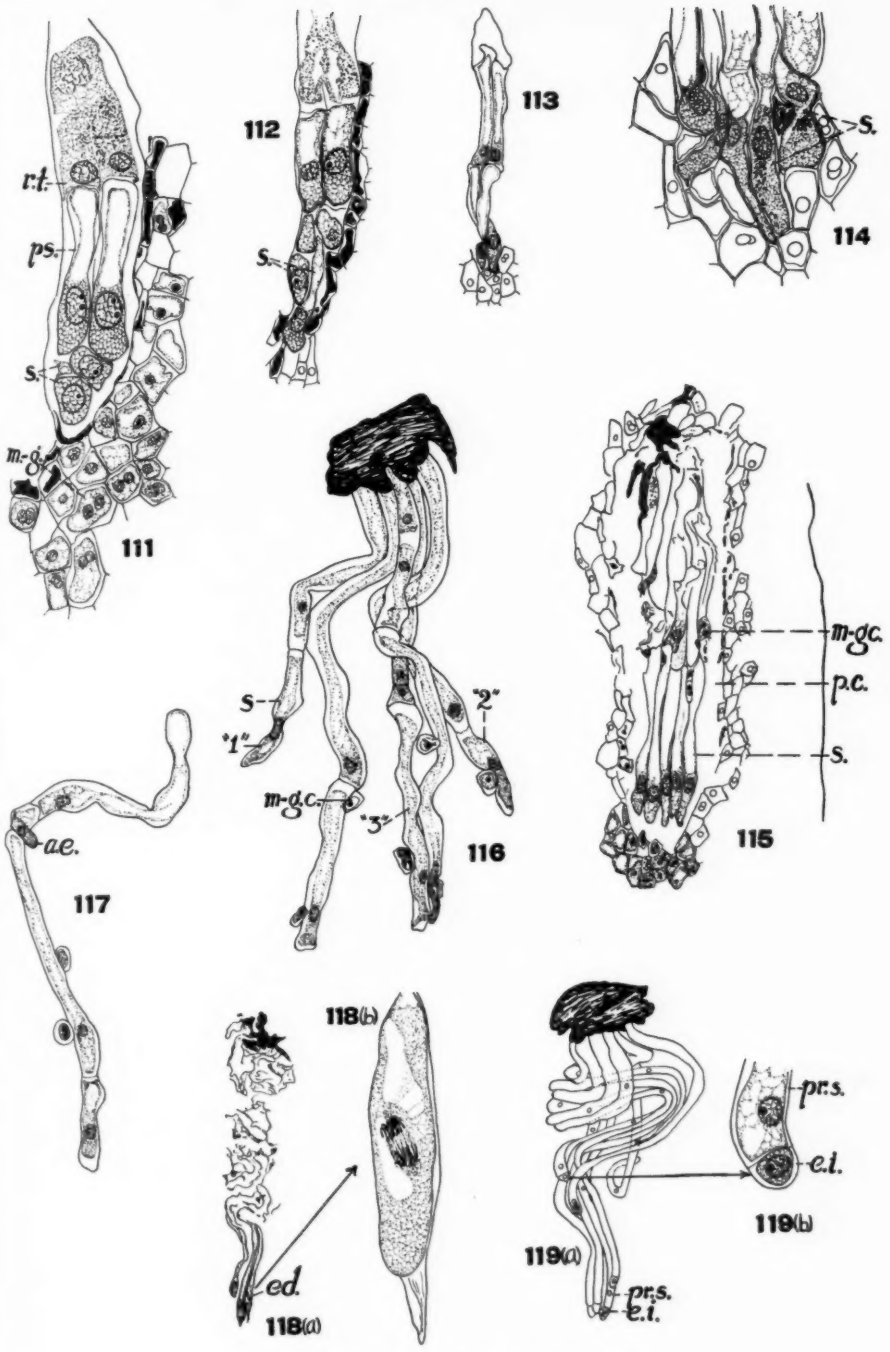


MATHEWS on JUNIPERUS VIRGINIANA

PLATE 7

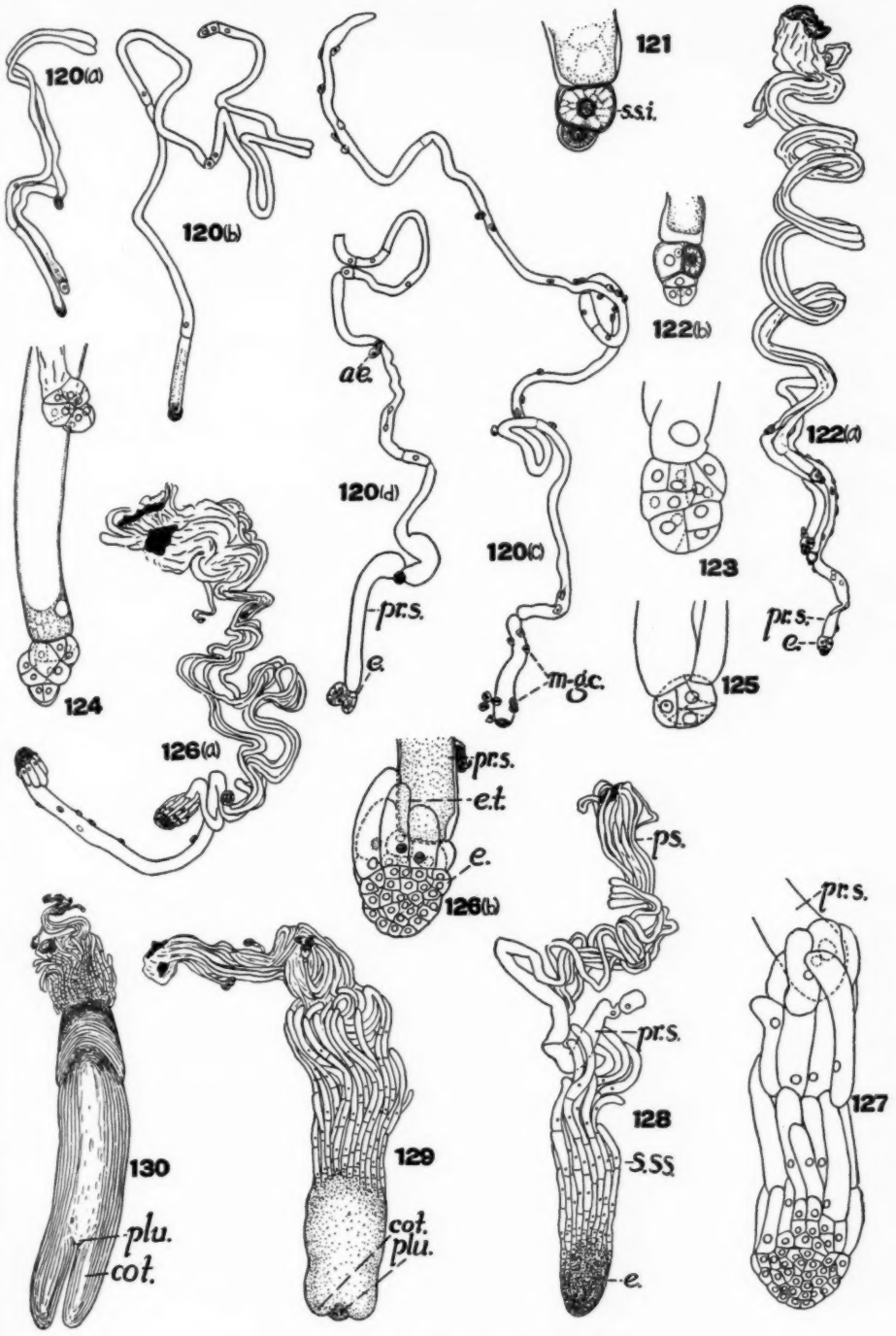


MATHEWS on JUNIPERUS VIRGINIANA



MATHEWS on JUNIPERUS VIRGINIANA

PLATE 9



MATHEWS on JUNIPERUS VIRGINIANA

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SEED DEVELOPMENT OF LOBELIA AMOENA*

By W. C. Hewitt

Plates 10–13

Historical Introduction

In 1878 Warming (42) published two figures of the young ovule of *Lobelia Dortmanna:* one with the megaspore mother cell about half grown and lying in almost a straight line with the funiculus, and with the primordium of the integument showing; the other of a nearly mature megaspore mother cell lying parallel with the funiculus, and the integument developed well beyond the outer end of the single-layered nucellus.

In the same year Vesque (39) gave the number of megaspores in *Lobelia laxiflora* as usually four but sometimes three. He also showed a mature egg apparatus of *Siphocampylus Manettiaeflorus* consisting of two elongated synergids and an egg cell, with a fusion nucleus just below, and an embryo sac 'tapetum' well developed.

In 1880 Ward (41) showed that the number of megaspores in *Lobelia syphilitica* is three, or four with the lower two lying side by side. Most of his paper is concerned with a 'cell-plate' which he showed passing transversely through the central vacuole of the two-nucleate embryo sac. None of his other figures showed such a plate. He showed figures of the mature sac both before and after the polar nuclei fuse to form the 'nucleus of the embryo sac'; and in his most mature figure shows the three antipodals disintegrating, but does not mention this in the text.

In 1881 Jönsson (17) described the development of the embryo sac in *Lobelia Dortmanna.* And for *L. Aubrietiae* he described the development of the megaspores from the megaspore mother cell, and described the mature sac. He also showed that the embryo sac develops from the lowest of the megaspores in *L. erinus.*

In 1882 Guignard (15) showed three megaspores for *L. erinus,* but said it is possible that a fourth develops; in any case it is the chalazal spore which develops into the embryo sac. He mentioned the early disap-

*A thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Botany.

pearance of the nucellus, and said that the upper end of the sac with the egg apparatus steals into the micropyle.

In 1896 Schlotterbeck (30) considered the transformed antipodals as playing a part in the formation of the chalazal haustorium of *Lobelia inflata*.

In 1901 Billings (5) showed a figure of the mature embryo sac of *Lobelia excelsa* with only the remnants of the antipodals. He described the synergids as being long with no nuclei (but had only one mature sac to study). He showed a well developed 'tapetum' extending from the lower end of the nutritive tissue to the upper tip of the embryo sac and showed a fibro-vascular bundle extending up into the funiculus. He described the early development of the endosperm as dividing first transversely, then by a longitudinal division in each of the resulting cells, the upper cells moving around the elongating egg cell which extends into the micropyle with them. He showed that the micropylar and chalazal haustoria each develop from two cells of the endosperm but did not show which cells develop into the large central mass of endosperm. For *L. Cliffordiana* he described the micropylar haustorium as developing from only the nuclei-bearing projections of the two upper cells and the chalazal haustorium as developing from a similar projection from a single lower endosperm cell.

In 1912 Armand (1) gave the haploid number of chromosomes as eight for *Lobelia erinus*, *L. urens*, and *L. Dortmanna*. He described the exine of the pollen for these species as having three pores of germination and germinating very easily in distilled water. He also described the grain as containing a granular protoplasm with a spherical vegetative nucleus rich in chromatin and an elongated generative nucleus surrounded by clearer protoplasm. He said that when the tube is 20 times as long as the diameter of the spore the vegetative nucleus disappears and the generative nucleus divides. These species show a single integument, very small nucellus, and a single hypodermal archesporial cell which gives rise to four megaspores, the lowest of which (sometimes the third) develops into the embryo sac. He described the embryo sac before the fusion of the polar nuclei and said that fertilization occurs in the usual way: the tip of the pollen tube touches the superior part of the embryo sac; the membranes in contact disappear; the synergids swerve; and the slightly elongated male gametes appear to enter them. He observed double fertilization, the polars not fusing until fertilization occurs. And according to him in these species, the fertilized egg elongates and divides transversely, the superior cell dividing transversely

to form a 4–8-celled suspensor, and the inferior cell dividing by three perpendicular walls to form eight cells which then divide by tangential walls to form a 16-celled embryo. He was not able to follow the development further but said that the mature embryo is small and is composed of undifferentiated cells.

In 1932 Rosen (28) described the development of the micropylar haustorium of *Lobelia Dortmanna* as developing from the upper daughter cells of the first two micropylar endosperm cells, and described the development of the endosperm as being cellular from the very beginning.

In 1935 Kausik (18), for *Lobelia trigona*, briefly described the development of the ovule, megaspores and embryo sac. The single layer of nucellus breaks down at the four-nucleate stage of the sac, the inner layer of the integument developing into a 'tapetum' which encloses the nutritive tissue at the chalazal end of the sac. The fusion nucleus is formed before fertilization. He described the divisions in the endosperm as being first transverse, followed by longitudinal divisions in each cell, the latter divisions being perpendicular to each other. The two lower cells develop directly into the chalazal haustorium; the two upper cells divide transversely, the upper daughter cells developing into the micropylar haustorium and the two lower daughter cells dividing to form the massive endosperm. He described the divisions of the embryo as being first transverse followed by three transverse divisions in each cell, making a four-celled embryo and a four-celled suspensor. He showed that the terminal cell of the four-celled embryo undergoes the next division by a longitudinal wall, followed by the division of the next cell also in the same plane. He said that the first three transverse divisions in the embryo cell can still be seen in late stages of the embryo, but has very few figures to illustrate this. He described in some detail the outer cells of the integument which become elongated and develop elaborate thickenings along their inner and lateral walls. In this paper he paid special attention to the nutrition of the embryo sac.

As far as seed development is concerned, the writer has found no mention of *Lobelia amoena* in the literature, and no work on the Lobeliaceae published in this country. In the literature cited above (except 1, 18) a brief account of one or more species of the Lobeliaceae was included in a general discussion of some particular phase of seed development. No full account of the seed development in any species of the family has been found.

The present work was done under the direction of Drs. W. C. Coker and H. R. Totten, and for this direction the writer is grateful. The

writer also wishes to express appreciation for helpful suggestions from Drs. J. N. Couch, Velma Matthews, and Vera Millsaps; and to Misses Alma Holland, Evelyn Mullen, and Constance Sherman for help in locating references in the literature and translating the articles.

MATERIALS AND METHODS

Material was collected around Chapel Hill, N. C., throughout September and October 1934; from Elizabeth City, N. C., in August 1935; from Chapel Hill during July and August 1937; and around Hartsville, S. C., during September, October, and November 1938. Material for sectioning was killed in weak chromo-acetic solution, formalin acetic alcohol, and a combination of Carnoy's and Nawaschin's fixing fluids. All of the best material was killed and fixed in the latter combination. The material was allowed to stand in Carnoy's for about ten minutes then changed to Nawaschin's fluid which was allowed to react over night.

The tissues were dehydrated through the ethyl alcohol series, and through the diethyl ether series. In nearly all cases the latter gave better results, but for the young embryo sac the alcohol series gave better results.

Haidenhain's iron-alum haematoxylin, Flemming's triple, and safranin-gentian violet stains were used. The safranin-gentian violet stain gave the best results.

Microspore mother cells and stages showing the divisions of these cells to form the microspores were killed and stained by Billing's iron acetic carmine technique. This gave good results on these stages.

The pollen grains and pollen tubes were stained by Feulgen's technique as described later in this paper.

Most of the drawings in this paper were made from material which was killed in a combination of Carnoy's and Nawaschin's fluids, dehydrated through the ether series, and stained with safranin-gentian violet. The drawings were made at table level with the aid of a Spencer camera lucida and reduced.

STRUCTURE OF THE FLOWER

The flower is considered epigynous although the top part of the ovary is not covered by any of the other floral parts. The stamens, petals, and sepals all arise from a common projection about midway between the top and bottom of the ovary. The anthers are joined together side by side forming a tube, but the filaments are separate. The petals

are fused together forming a long tube which is two-lipped. The sepals are fused together at the very bottom with the upper parts projecting as five long narrow lobes. The pistil is compound, being composed of two locules, a long style, and a two-lobed stigma. Before the lobes of the stigma open to expose the stigmatic surface, the outside of the young stigma is covered with long bristles which brush the pollen out of the pollen sacs as the stigma grows up through the anther tube. The placenta is axial with each locule containing a large lobe which is covered with numerous anatropous ovules.

MICROSPORANGIUM AND DEVELOPMENT OF MALE GAMETOPHYTE

The general appearance of the anther in cross section is typical. There are four microsporangia, each containing a mass of microspore mother cells surrounded by four rows of wall cells (Fig. 1).

The microspore mother cells are cuboidal, containing a distinct nucleus and small vacuoles. Later the contents of these cells become more homogeneous; the vacuoles disappear; and the nucleus gets larger. The two divisions of the mother cell are in rapid succession, the second division occurring before the spindle of the first is gone (Figs. 2–4); and both nuclear divisions occurring before wall formation begins (Fig. 5). When the young spores are first formed most of the cell is filled with a large vacuole, the spore at this stage resembling a typical fat cell with only a very narrow band of cytoplasm around the outside of the vacuole (Fig. 6). On one side of the vacuole the cytoplasm is a little broader, and it is here that the nucleus is located. Similar vacuolated young microspores have been described by Locke (20) for *Asimina triloba* and by Madge (21) for *Viola odorata*. As development continues the entire cell becomes filled with rather granular cytoplasm with tiny vacuoles scattered throughout (Fig. 7). The microspore then divides in the usual way to form two cells of unequal size (Fig. 8). However, this division often occurs while the microspore nucleus is on one side of the cell with only a few bands of cytoplasm crossing the large vacuole. The smaller cell, the generative cell, is located on one side of the spore when it first appears and is separated from the tube cell by a distinct membrane. Soon the generative cell moves away from the outside of the spore and becomes entirely surrounded by the contents of the larger tube cell (Fig. 9). The membrane of the generative cell still surrounds its cytoplasm after moving into the vegetative cell, but this membrane disappears very early. The nucleus of the generative cell has the appearance of a resting nucleus

for only a short time, soon becoming oblong in shape and smaller in size, with the nucleolus disappearing and large chromatin granules appearing which are scattered throughout the nucleus. When first formed the cytoplasm of the generative cell is not very dense, having numerous small vacuoles scattered throughout its contents. After moving into the center of the pollen grain this cytoplasm changes into a dense spindle-shaped mass surrounding the nucleus and projecting at each end of the nucleus. This leaves a space surrounding the dense cytoplasm that does not contain any granules and does not stain (Fig. 9). When the cell membrane of the generative cell disappears, the granular cytoplasm of the vegetative cell moves in until it touches the dense cytoplasm of the generative cell; but the cytoplasm of the generative cell is denser and still can be recognized as a spindle-shaped mass until the formation of the pollen tube begins (Fig. 10).

When this change is taking place in the generative cell, the nucleus of the vegetative cell is disappearing, the nuclear membrane disappearing first. Later the entire nucleus including the nucleolus disintegrates, leaving for a time small fragments of deeply staining material scattered in the cytoplasm of the cell. The tube nucleus usually entirely disappears before the pollen tube begins to develop, but in a few cases a nuclear mass about the size of the nucleolus of the tube nucleus was seen in the pollen grain with the generative nucleus as the tube began to develop. In unusual cases one or more fragments from the tube nucleus could be distinguished in the tube; however at this time they stained very little darker than the cytoplasm of the tube.

As early as 1879 Elfving (13) thought that the tube nucleus degenerated before the germination of the pollen grain in *Hypericum calycinum*. The degeneration of the tube nucleus in the pollen grain has been reported by Shattuck (33) 1905, for *Ulmus americana*; by Poddubnaja-Arnoldi (26) 1927, for *Echinops sphaerocephalus*, *Carduus crispus*, and *Centaurea scabiosa*, and (27) 1933, for *Senecio platanifolius*; by Sax and Edmonds (29) 1933, for *Tradescantia*; and by Cooper (11) 1938, for *Pisum sativum*. The tube nucleus has been reported to show signs of disintegration either in the mature pollen grain or early in the tube by Osterwalder (25) 1898, for *Aconitum napellus*; by Barnes (3) 1885, for *Campanula americana*; by Armand (1) 1912, for three species of *Lobelia*; by Wylie (44) 1923, for *Vallisneria spiralis*; by Finn (14) 1928, for *Vinca minor*; by Madge (21) 1929, for *Viola odorata*; by Cooper (9) 1935, for *Chenopodium hybridum*, *C. album*, *Atriplex patula* var. *hastata*, and *Salsola kali*. There are a large number of cases which

show some evidence of a degenerating tube nucleus, and the writer is of the opinion that this degeneration is more common than is often believed. Wulff and Maheshwari (43) express some doubt as to whether in reported cases the tube nucleus does actually disappear or whether its apparent absence may be due to failure to stain. We have no doubt that in the case of our plant, the disappearance of this nucleus is actual and not merely apparent.

However, in the numerous botanical textbooks that have been examined during this study, including the latest ones on cytology, no mention is made of the not-so-infrequent early disappearance of the pollen tube nucleus in angiosperms, and it is often stated that this nucleus is supposed to direct the growth of the pollen tube. The full development of the pollen tube in the complete absence of the pollen tube nucleus brings up the interesting theoretical question of the ability of generative nuclei to function vegetatively as well. This is of course assuming that the influence of a nucleus is needed for vegetative growth.

To sum up, at the time that the pollen grain of *L. amoena* is shed it usually contains a generative nucleus surrounded by a definite mass of cytoplasm and only fragments of the tube nucleus, if there is any of it remaining at all. In the pollen tube it is only in exceptional cases that even obscure fragments that might have come from the degenerating nucleus, can be found. Its wall consists of two layers, a heavy exine and a very thin intine. Three elongated pores have developed in the exine, where the intine projects outward beyond the general surface of the spore (Fig. 10).

The pollen grains germinate very easily in a 5% sugar solution or in distilled water, but in distilled water the tubes do not grow long before they burst. In either case the germination begins almost at once. As soon as the tube projects through the exine, the cytoplasm on the inside can be seen in constant motion, moving from the spore into one side of the tube, up the other side of the tube and back into the spore. By the time that the tube grows in length equal to about twice the diameter of the spore, the contents within the spore itself become so thin that their movements are visible. A similar streaming of the cytoplasm in the young tube was described by Barnes (3) for *Campanula americana*. The contents of the spore become very vacuolated, the vacuoles soon extending down into the tube.

For detail study of the pollen tubes, the grains were germinated and stained according to the directions given by Lee (19). A thin coat of

melted agar-sugar solution (1.5 grams agar, 5 grams cane sugar, 100 cc. distilled water) was smeared on the slide, and the pollen grains were sifted into the solution before it hardened. They were germinated in a moist chamber from 2–15 hours. These tubes were then stained by the Feulgen's technique.

The generative nucleus does not pass into the tube until the tube has grown longer than the diameter of the grain. At the time this nucleus enters the tube, there is usually no distinct mass of cytoplasm surrounding it (Fig. 11). The generative nucleus divides to form the two oblong or irregular-shaped male nuclei in the tube after the tube is very long (Fig. 13), this division occurring when the generative nucleus is about the same distance from the tip of the tube as is shown in figure 12.

DEVELOPMENT OF THE OVULES

The ovules begin as little protuberances on the surface of the placenta, and up until the time of bending the entire ovule is an undifferentiated mass. Very early, however, a single hypodermal cell enlarges and reacts differently to the stains. This cell, identified as the archesporial cell, becomes very conspicuous and functions directly as the megaspore mother cell, which is the usual procedure for the Sympetalae (12). When the mother cell is first recognized, the integument has already begun developing on the outer side of the curving ovule (Fig. 14). The mother cell is covered by only one layer of nucellus. This cell elongates to two or three times it original size before its first division (Fig. 15).

MEGASPORES

The megaspore mother cell divides transversely to form a dyad (Fig. 16); then each of these cells divides in the same plane to form a linear tetrad of spores (Fig. 17). It was not determined which of the divisions is the reduction, but the megaspores themselves contain the haploid number of chromosomes, seven in this species. In the usual way the three spores nearest the micropyle soon disappear while the lower one grows larger (Figs. 18, 20). Although this is the usual procedure, a few cases have been found in which only three megaspores are formed, the upper cell of the dyad not dividing to form two spores but containing two nuclei (Fig. 19). In these cases the spore which elongates to form the embryo sac is the one farthest from the micropyle. In one case the second and fourth spores, counting from the micropyle, were both dividing to form an embryo sac (Fig. 21). The second spore

was ahead, having already formed a two-nucleate sac with the nuclei in opposite ends of the sac while the nucleus of the fourth spore was still in the telophase stage of mitosis. The collapsed first and third cells could be easily seen. No other stages in such a development were found.

THE EMBRYO SAC

The development of the embryo sac follows the monosporic 8-nucleate normal-type described by Maheshwari (22) and other recent writers. The chalazal megaspore, containing a vacuole in each end, enlarges until it is as large as the four original spores before it begins to divide (Fig. 20). As soon as the first division is complete one nucleus moves to each end of the sac, and the vacuoles change from the ends to a single large vacuole in the center (Figs. 22, 23). Figures 24 and 25 show four- and eight-nucleate sacs. Soon strands of cytoplasm cross the large central vacuole of the sac, and three cells are cut off at each end, leaving the two polar nuclei in the large central part. The three antipodals look almost like an inverted egg apparatus when they are fully developed, but they disappear very early. Figure 26 shows the egg apparatus (only one synergid in this section) at the upper end of the sac, and the three antipodals at the lower end. The two polar nuclei move towards each other and fuse in the upper end of the sac. In this fusion the nucleoli remain distinct for some time after the nucleoplasm mixes (Figs. 27, 28). When the egg apparatus is mature the antipodals are disappearing (Fig. 29). At this time each of the synergids is much longer than wide and contains a large vacuole which fills the lower half of the cell, the nucleus lying in the upper half close to the vacuole. The micropylar ends of the two synergids break through the embryo sac and push up into the micropyle. The egg cell is much shorter than the synergids; and its lower half, containing the densest cytoplasm, extends below the synergids. Figure 30 shows a cross section of the synergids above their nuclei; here neither the egg cell nor the sac is present.

NUCELLUS

The part of the nucellus which covers the sporogenous tissue is never more than one layer thick. The cells of this layer are cuboidal when first observed and in general give the appearance of being in a healthy condition (Fig. 14). Soon after the megaspore mother cell begins to elongate, the cells of the nucellus stop multiplying, become

almost void of cytoplasm, and give the appearance of being stretched (Figs. 15–20). The four megaspores are not much larger than the large mother cell which gave rise to them, and the functional megaspore does not exceed the size of the four original megaspores until its first division. The two-nucleate sac increases in size to about one and a half times the size of the four original spores. It is during this increase in size that the sac splits the nucellus at its upper end, and the upper end of the two-nucleate sac moves out into the space surrounded by the integument (Fig. 22). Bushnell (6) has described a similar change in the appearance of the embryo sac of *Monarda fistulosa* following the first division of the sac nucleus, and she has shown that the two-nucleate sac breaks through the nucellus. The layer of nucellus which originally surrounded the upper end of the mother cell and the four spores rapidly disappears, and by the time that the sac is mature it is entirely gone. The nucellus (nutritive tissue) at the lower end of the embryo sac is present until the endosperm is formed. The endosperm forces its way down into this part of the ovule and develops into a large haustorium which absorbs all of the nucellus before the zygote divides.

INTEGUMENT

There is a single integument, the growing point of which may be seen at the time when the ovule first begins to bend. The integument is first seen on the side of the ovule farthest from the funiculus (Fig. 14). The cells of the very young integument are large; as they increase in number, they get smaller (Figs. 14, 15, 17). The integument grows rapidly. By the time the mother cell is mature, the integument has grown out beyond the nucellus at the upper end (Fig. 15); and by the time the functional megaspore is enlarging the integument has grown out beyond the nucellus so far that the upper end of the nucellus seems to lie about midway between the upper and lower ends of the ovule. The integument continues its development until the endosperm is formed.

'TAPETUM'

The inner layer of cells of the integument becomes distinguished as a 'tapetum' as early as the megaspore stage, even before the three micropylar megaspores begin to disappear (Fig. 17). At the time that the functional megaspore is ready to divide, the 'tapetum' is well developed from the place where the integument joins the nucellus at the lower end to a few cells beyond the upper end of the nucellus (Fig. 19).

As soon as the nucellus disappears, the 'tapetum' lies next to the embryo sac. The 'tapetum' reaches its maximum development about the time of fertilization. At this stage the cells of the 'tapetum' are long and narrow with their longitudinal axis perpendicular to the longitudinal axis of the sac. At this stage the tapetal cells contain large nuclei which do not stain as clearly as the other nuclei of the integument, but the rest of the tapetal cell stains much darker than the surrounding cells of the integument. The cells of the 'tapetum' increase in number from the time that they first appear until the time of fertilization, additional cells being cut off in the 'tapetum' and new cells being added on at the micropylar end. But this increase in length of the 'tapetum' does not keep pace with the rapid increase in length of the developing embryo sac; therefore the upper end of the mature sac projects out beyond the 'tapetum.' The part of the embryo sac which increases in width is the part which is not surrounded by 'tapetum' (Fig. 26). From the appearance of the cells of the integument surrounding the upper end of the sac above the 'tapetum,' the sac digests its way up into the micropyle. As soon as the endosperm begins forming, the entire 'tapetum' begins collapsing (Figs. 38–40).

FERTILIZATION

The pollen tube grows down through the micropyle, destroys one of the synergids, and discharges its contents into the embryo sac. One of the male nuclei was seen in different stages of fusion with the fusion nucleus (Figs. 33, 34), and the other male nucleus was seen fusing with the egg nucleus (Fig. 32). Usually the two polar nuclei fuse before fertilization, but often this fusion is delayed until fertilization; then one male nucleus and the two polars fuse at the same time (Fig. 31). In both the endosperm nucleus and the zygote nucleus, the nucleoli remain distinct for some time after the fusion of the nucleoplasm (Figs. 33–36). Johansen (16) has described a similar fusion of the gametes of *Clarkia elegans*. The chromosome number in the cells of the integument is 14, and the number in the cells of the endosperm is around 20. These figures are approximately twice and three times the haploid number, seven, counted in the embryo sac.

ENDOSPERM

The endosperm nucleus divides before the sperm and egg nuclei completely fuse (Fig. 35). The first division of the endosperm is perpendicular to the longitudinal axis of the sac. A cell wall is formed

after the first division of the nucleus, and there are no free nuclear divisions in the endosperm. The next division is longitudinal in each of these cells with the cell walls in the two cells almost perpendicular to each other. Figures 36 and 37 were made from ovules cut obliquely so that all four of the cells were showing. In many cases only one cell of either the lower two or upper two shows in a single section (Figs. 38–40). Now each of these four cells divides transversely forming four tiers of two cells each (Fig. 37). The two cells of the upper tier never divide again but move up around and beyond the zygote and force their way up into the micropyle, destroying what is left of the egg apparatus except the egg (zygote), and forming a large micropylar haustorium (Figs. 38–40). The two cells of the lowest tier do not divide again either, but move down into the chalazal end of the ovule, destroying the nutritive tissue, and developing into a large chalazal haustorium (Figs. 38–40). Cross sections of mature haustoria, both micropylar (Fig. 42) and chalazal (Fig. 41), show that they are composed of these two original cells. The cells of the two middle tiers multiply rapidly to form the massive endosperm of the seed (Figs. 38–40). About twenty cells are formed in the endosperm and the two endosperm haustoria are rather well developed before the first division of the zygote (Fig. 38).

The origin of the chalazal haustorium and the central mass of endosperm as described above is not in agreement with the account given for *Lobelia trigona* by Kausik (18), who is the only writer having discussed the origin of these structures for the Lobeliaceae. The origin of the micropylar haustorium is, however, in agreement with the account given by Rosen (28) and Kausik (18).

EMBRYO

The first division of the zygote is transverse, cutting off what later proves to be a primary embryonal cell *e* and a primary suspensor cell *s* (Fig. 44). In all cases the suspensor cell is the first of these to divide; this is also a transverse division (Fig. 45) cutting off a middle cell *m* and a basal cell *l*. Often these two cells will divide again by transverse walls before the embryo cell divides; but the most common procedure is for the embryo cell to divide first by a transverse wall (Fig. 46) cutting off an apical cell *a*, which does not divide again until longitudinal divisions begin, and a second embryonal cell *f*. The cells *m*, *l* divide by transverse walls forming a four-celled suspensor *n*, *o*, *p*, *h* (Figs. 47–49). Each of these suspensor cells may divide again, the divisions

in the distal end being either transverse or longitudinal, producing a suspensor *s* about 8–12 cells in length (Figs. 50–55). The second cell *f* of the embryo divides by a transverse wall to form cells *b*, *g* (Figs. 48, 49). The cells *a*, *b* next divide by longitudinal walls, and the cell *g* divides by a transverse wall. Either of these cells may be the first to divide, but the product is always the same: two cells side by side in both *a*, *b* and two superimposed cells *c*, *d* formed from *g* (Figs. 49, 50). The first vertical divisions in *a*, *b* are perpendicular to each other (Fig. 50). The embryo now consists of four tiers of cells *a*, *b*, *c*, *d*, and these four tiers can be traced throughout the development to the mature embryo (Figs. 50–56). The next division in both *a*, *b* is vertical and perpendicular to the first division in that tier (Fig. 51). Cell *c* divides by a vertical wall (Fig. 51). This produces two tiers of four cells each at the distal end followed by a tier of two cells *c* and a tier of one cell *d*.

In the distal tier *a*, anticlinal divisions occur first (Fig. 52) followed by periclinal divisions (Fig. 53) which cut off the dermatogen in this region of the embryo. The first anticlinal divisions in this tier can be traced in the development to the mature embryo. These anticlinal divisions separate the future cotyledons from the future plumule. The outer end of one of these divisions is marked by *x* in figures 52–56.

The next division in tier *b* is tangential, cutting off the dermatogen one stage earlier than in tier *a* (Fig. 52). The inner cells divide again longitudinally separating the future periblem from the future plerome (Fig. 53). Both longitudinal and transverse divisions occur in the future development of the periblem and plerome in this tier (Figs. 54–56). A heavy line separates the periblem from the plerome in figure 56.

Tier *c* develops into a single semi-circular layer of cells at the base of the embryo (Figs. 52–56). This layer helps to complete the periblem, dermatogen, and root cap of that region.

The single cell of tier *d* does not divide until the embryo is rather well developed; then it divides first by a transverse wall (Fig. 54). Both the proximal and distal cells from this division divide by longitudinal walls (Fig. 55). The cells of the proximal row do not divide again, but the cells of the distal row divide transversely (Fig. 56), separating a periblem portion from a dermatogen portion. The cells of the proximal row become a part of the root cap, which is increased on all sides by a single cell from tier *c* and in some places by extra cells cut off from the dermatogen of tier *b*. At the base of the mature em-

bryo there are one layer of root cap, one layer of dermatogen, and two layers of periblem (Fig. 56).

The basal cell of the suspensor extends into the micropylar haustorium and evidently functions as an embryonal haustorium (Figs. 44–50, 36–40). The two cells of the micropylar haustorium have grooves along their inner surfaces into which the embryonal haustorium fits (Fig. 42). The embryonal haustorium collapses before the seed is mature.

The development of the embryo as described here is in close agreement with the brief description given by Kausik (18) for *Lobelia trigona*. He did not make it clear where the three transverse divisions occur in the primary embryonal cell, and the plane of division for the first longitudinal division in cells *a* and *b* is not in agreement with that found in the present work. The development of the embryo as described by Armand (1) for three species of *Lobelia* agrees with the *Capsella*-type, which resembles the type discussed here only in the fact that it has a long suspensor. The differences between the two types of development lie in (1st.) the portion of the embryo derived from the distal cell following the first transverse division of the zygote, (2nd.) the formation of the octant stage, (3rd.) the plane of the first division in the distal cells of the octant stage, and (4th.) the amount of suspensor formed from the hypophysis. Cell *g* (Fig. 49) is considered the hypophysis in the present work.

The writer is of the opinion that there are as many examples showing some degree of relationship with the type of development described here as there are showing relationship with the *Capsella*-type. In very few papers are all of these points brought out, but some relationship is shown with this type of development in *Senecio aureus* described by Mottier (24); in *Erechtites hieracifolia* by Cooper (10); in *Silphium* by Merrell (23); in *Ulmus fulva* by Walker (40); in *Daucus carota* by Borthwick (4); in *Pontederia* by Smith (34); in *Rhytidophyllum* by Cook (7); in *Carum carvi* by Souèges (38); in *Sherardia arvensis* by Souèges (37); in *Myosurus minimus* by Souèges (35); in *Senecio* by Souèges (36); and in *Melilotus* by Cooper (8).

THE MATURE SEED

One layer of cells on the outside with a few extra cells around the haustoria make up the seed coat. The remainder of the integument has been absorbed by the endosperm. The cell walls of this outer layer have developed a thick band in the form of a ring on the four

lateral sides of the cell, touching neither the base nor the outer edge of the cell. A few of the outer cells at the micropylar end do not develop this thickened ring around the cell. The embryo is small, and most of the old suspensor is still attached to it. A large part of the mature seed is made up of endosperm, including the two big haustoria. The food material has been absorbed from the endosperm adjacent to the embryo, leaving around the embryo a large clear space which stains very little; the remainder of the endosperm stains readily (Fig. 43).

SUMMARY

1. In *Lobelia amoena* four microspores develop from a microspore mother cell, the cell plates forming after the second maturation division.

2. In the microspore, a small generative cell is cut off on one side, but later it moves into the tube cell where its cell membrane and then its cytoplasm disappear.

3. The tube nucleus disintegrates before the pollen tube begins to develop.

4. The generative nucleus divides in the tube to form the two male nuclei.

5. There are numerous anatropous ovules in each ovary.

6. A single hypodermal archesporial cell functions as the megaspore mother cell, usually producing four (sometimes three) megaspores, the chalazal one of which is functional.

7. The development of the embryo sac follows the monosporic 8-nucleate normal-type.

8. The antipodals begin disappearing by the time the sac is mature.

9. The mature synergids break through the micropylar end of the sac.

10. The single layer of nucellus breaks down following the first division of the functional megaspore.

11. The inner layer of cells of the massive integument develops into a conspicuous 'tapetum' which is present from the megaspore stage until the endosperm develops.

12. The polar nuclei fuse either before or during fertilization.

13. Double fertilization occurs.

14. Endosperm development is cellular; two cells from each end of the eight-celled endosperm develop into the large micropylar and chalazal haustoria.

15. The first division of the embryo cuts off a primary embryonal cell and a primary suspensor cell, the latter developing into an 8–12-

celled suspensor, the upper cell of which is embedded in the micropylar haustorium and functions as an embryonal haustorium.

16. The embryonal cell divides transversely, the proximal daughter cell dividing by two transverse walls forming four tiers of cells in the embryo which can be traced in the development to the mature embryo.

17. The octant stage is formed by two longitudinal divisions in both the distal and subdistal cells of the proembryo.

18. The first divisions in the four distal cells of the octant are anticlinal and separate the future cotyledons from the future plumule.

19. The first divisions in the four cells next to the distal tier of the octant stage cut off the dermatogen of that region, and the second divisions separate the future plerome from the future periblem.

20. All the derivatives from the primary embryonal cell develop into parts of the embryo; all the derivatives from the primary suspensor cell become part of the suspensor.

21. The seed coat consists of a single layer of cells whose lateral walls have become thickened.

22. Most of the mature seed is made up of endosperm, including the two large haustoria. The mature embryo is small.

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EXPLANATION OF THE PLATES

The figures are arranged so that the micropylar end of the ovule is turned towards the bottom of the page.

PLATE 10

Fig. 1. Cross section of a young anther showing four layers of wall cells surrounding a mass of microspore mother cells. × 780.
Fig. 2. Microspore mother cell in early prophase of first maturation division. × 780.
Fig. 3. Microspore dyad, just after first maturation division. × 780.
Fig. 4. Three of the four microspore nuclei just after the second maturation division. × 780.
Fig. 5. Wall formation between the microspores after the second maturation division. × 780.
Fig. 6. Young microspore, freed from mother cell, showing a thin band of cytoplasm surrounding a large central vacuole. × 780.
Fig. 7. Slightly older microspore with a number of small vacuoles instead of the one large vacuole. × 780.
Fig. 8. Microspore with generative and tube cells. × 975.
Fig. 9. Generative cell surrounded by cytoplasm of tube cell. Part of the contents of the generative cell do not stain. The tube nucleus is beginning to disintegrate, i.e. the nuclear membrane is breaking down. × 780.
Fig. 10. Mature pollen grain showing three pores in the exine. The generative nucleus is surrounded by a small mass of deeply staining cytoplasm. There are three small bodies in the cell which appear as the tube nucleus disappears. × 780.

Fig. 11. Microspore with young pollen tube, oblong generative nucleus. × 780.
Fig. 12. More mature pollen tube showing generative nucleus in the tube. × 780.
Fig. 13. Portion of mature pollen tube showing two male nuclei. × 780.
Fig. 14. Longitudinal section of a young ovule showing a single hypodermal archesporial cell, a single layer of nucellus, the beginning of a single integument, and a funiculus. × 780.
Fig. 15. Longitudinal section through an older ovule, showing mature megaspore mother cell surrounded by nucellus and integument. × 780.
Fig. 16. Longitudinal section through nucellus, showing dyad formed during first maturation division of megaspore mother cell. × 780.
Fig. 17. Longitudinal section through nucellus and portion of surrounding integument showing four linear megaspores and the beginning of a 'tapetum.' × 780.

PLATE 11

Fig. 18. Similar to Fig. 17, showing chalazal megaspore developing at the expense of the other three. × 780.
Fig. 19. Similar to Fig. 17. In this case the nuclear division of the micropylar cell of the dyad was not followed by cytoplasmic division. There are only two megaspores to disintegrate. × 780.
Fig. 20. Older stage, same plane, showing three collapsed micropylar megaspores, and one large chalazal megaspore with a vacuole at each end. × 780.
Fig. 21. Longitudinal section through megaspores showing the second and fourth megaspores, counting from the micropylar end, developing into embryo sacs while the first and third are disintegrating. × 780.
Fig. 22. Longitudinal section through ovule showing two-nucleate embryo sac already broken through the nucellus at the micropylar end, showing a vacuole in the center of the sac, a large integument, and a well developed 'tapetum' surrounding the nutritive tissue at the chalazal end of the ovule. × 388.
Fig. 23. Two-nucleate embryo sac similar to one in figure 22. × 780.
Fig. 24. Longitudinal section of four-nucleate embryo sac. A small vacuole has appeared at the chalazal end of the sac. × 780.
Fig. 25. Longitudinal section of eight-nucleate sac, with small vacuole at each end of the sac. × 780.
Fig. 26. Longitudinal section of an older ovule showing a well developed integument and 'tapetum,' the old nucellus, three well developed antipodal cells, two polar nuclei, one synergid, and an egg cell. The other synergid was cut off in the adjacent section. × 337.
Fig. 27. Two polar nuclei touching. × 780.
Fig. 28. Two polar nuclei in the process of fusion; the nucleoplasm has fused, but the nucleoli have not. × 780.
Fig. 29. Mature embryo sac ready for fertilization, showing two synergids, egg cell, fusion nucleus, and three disintegrating antipodal cells. × 780.
Fig. 30. Cross section of synergids above their nuclei. × 780.
Fig. 31. Triple fusion of two polar nuclei and male nucleus. × 780.
Fig. 32. Egg nucleus and male nucleus uniting; the male nucleus is smaller and stains lighter. × 780.

PLATE 12

Fig. 33, 34. Male nucleus uniting with fusion nucleus. The nucleoli may remain distinct for some time after the nucleoplasm fuses. × 780.

Fig. 35. Longitudinal section through the embryo sac just after fertilization, showing the first division of the endosperm nucleus; the egg cell, the nucleus of which contains the nucleoli of the egg and male nucleus, the nucleoplasm already fused; the pollen tube, which has destroyed one of the synergids; and the other synergid still in a normal condition. × 780.

Fig. 36. Longitudinal section through an older ovule, showing 'tapetum'; four cells in the endosperm, the two micropylar cells pushing up around and beyond the zygote, which has elongated but not divided; the nucleoli still distinct in the zygote nucleus. × 337.

Fig. 37. Section showing transverse division of cells in the four-celled endosperm. × 337.

Fig. 38. Similar to fig. 36, slightly older, showing micropylar haustorium forming from two micropylar cells of the endosperm, embryonal haustorium within the micropylar endosperm haustorium, the embryonal haustorium at this stage being merely the micropylar end of the much elongated zygote; and a chalazal haustorium developing from two chalazal cells of the endosperm at the expense of the nutritive tissue in the chalazal end of the ovule. × 172.

Fig. 39, 40. Longitudinal sections of older ovules showing the elongation of the ovule accompanied by the multiplication of the cells of the endosperm and the stretching of the cells of the integument; the enlarging haustoria; and the developing embryo. × 172.

PLATE 13

Fig. 41. Cross section of chalazal haustorium. × 172.

Fig. 42. Cross section of micropylar haustorium. × 172.

Fig. 43. Longitudinal section of mature seed, showing seed coat made up of scarcely more than a single layer of cells; the massive endosperm still attached to the two haustoria; the embryo with suspensor collapsing. The embryo has used the food material in the endosperm cells all around it. × 78.

Fig. 44–56. Longitudinal sections of embryos. For explanations see the text. Figure 56 is a section of a mature embryo. × 337.

PLATE 10

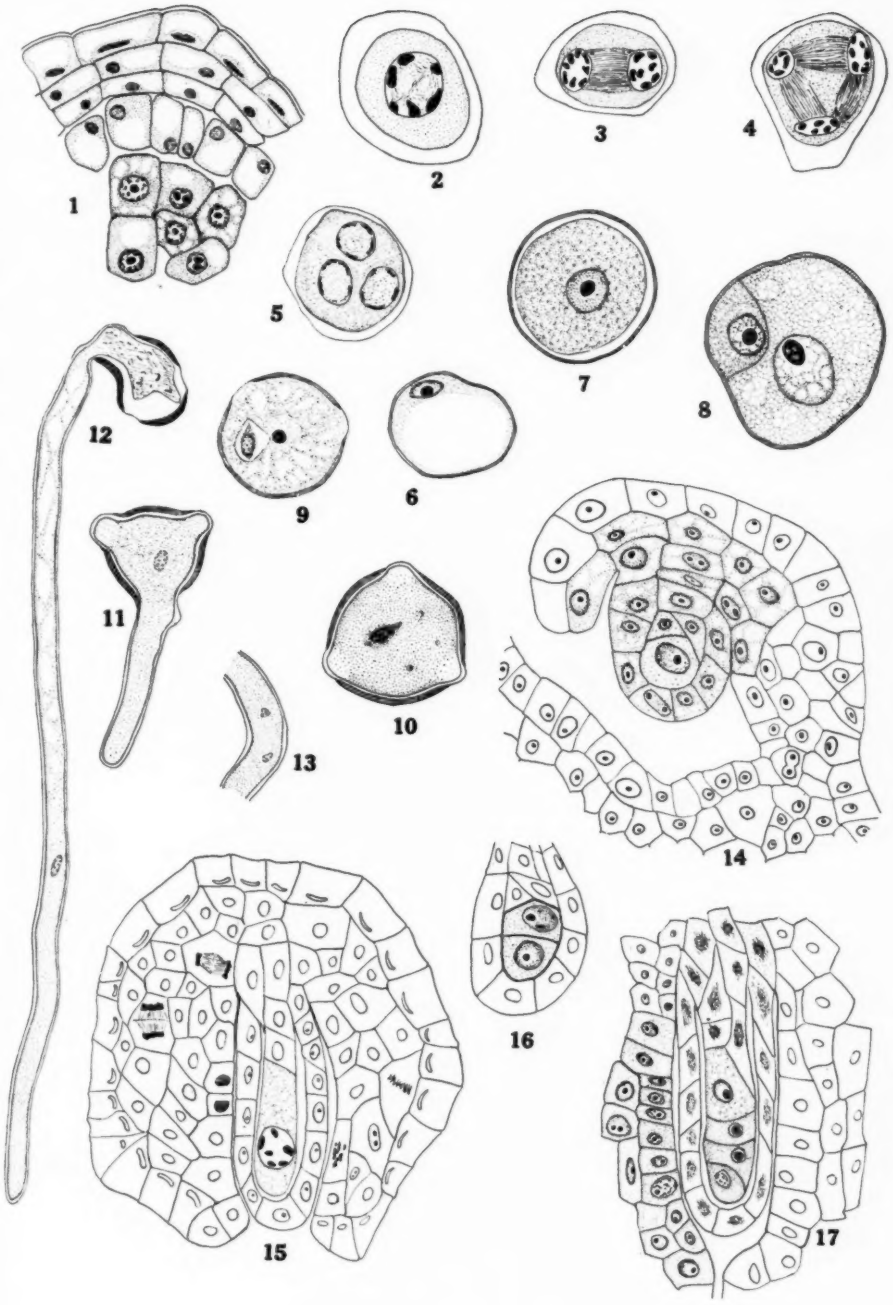

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PLATE 11

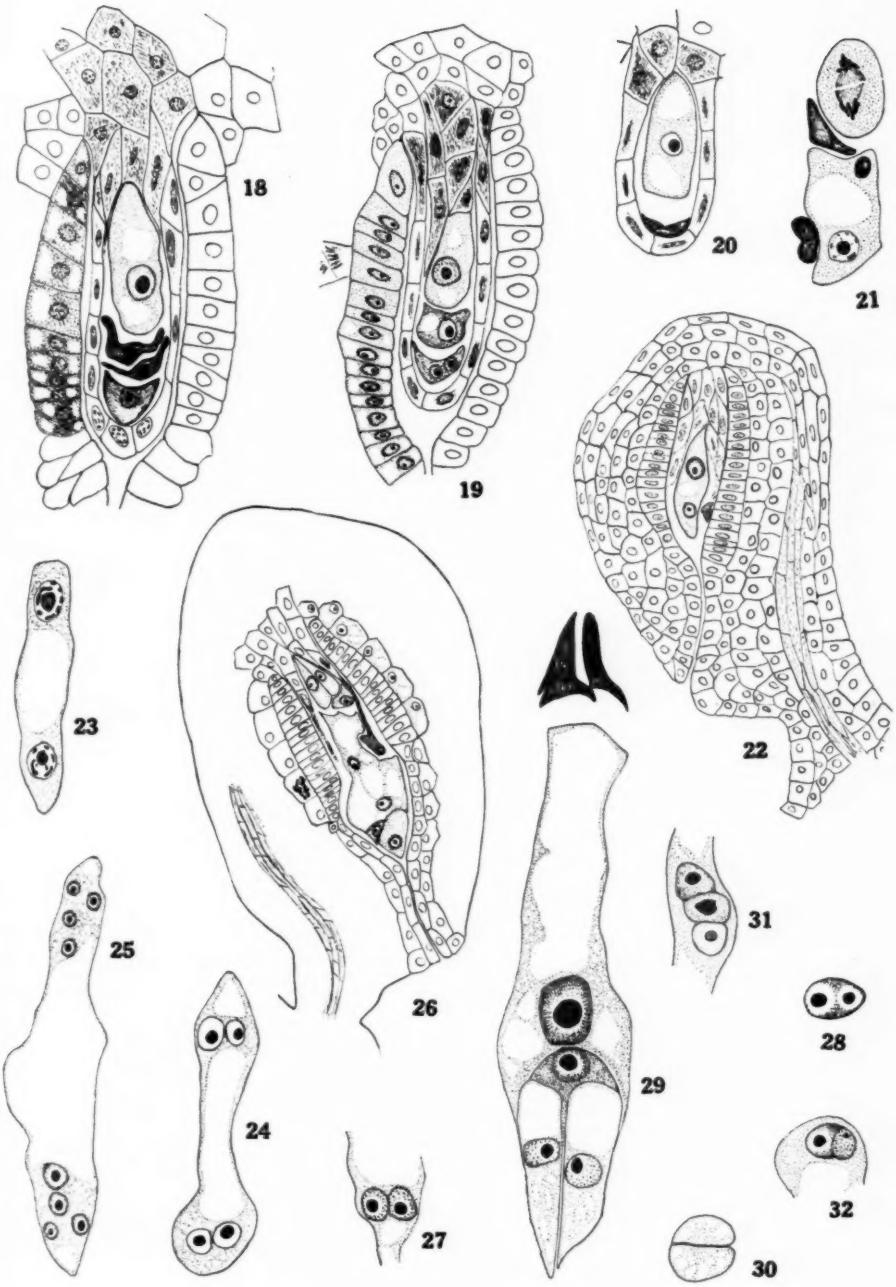

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PLATE 12

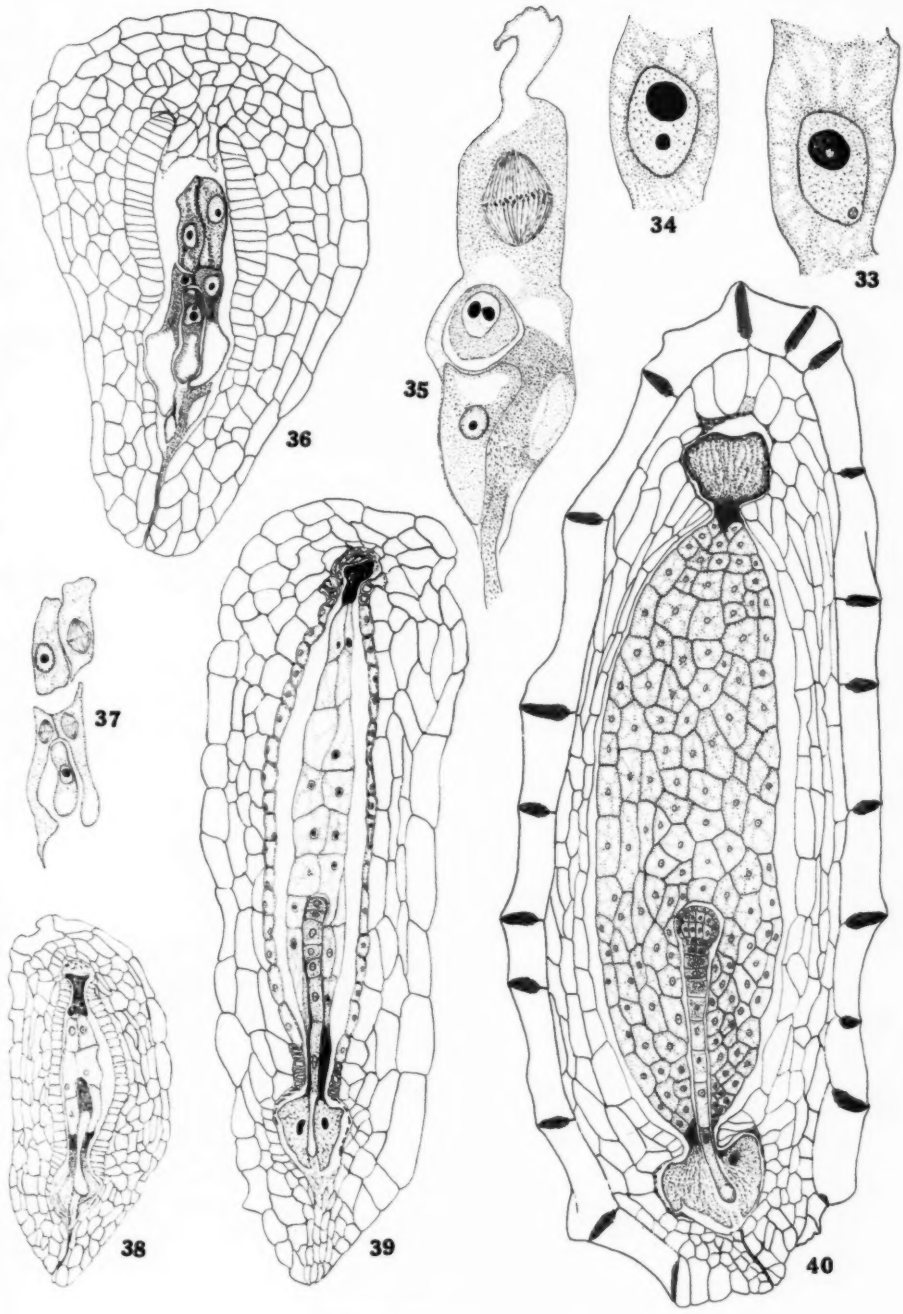
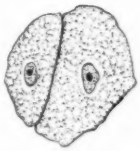


PLATE 13



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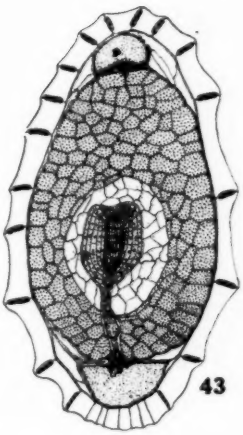

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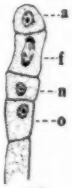

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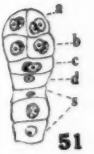

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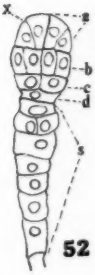

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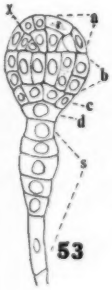

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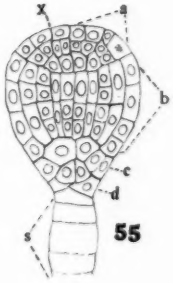

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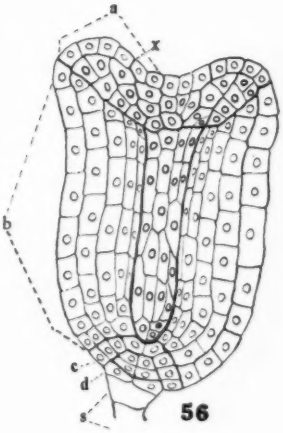

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FRESHWATER ALGAE OF NORTH AND SOUTH CAROLINA. PART I. CYANOPHYCEAE*

By Paul J. Philson

Plates 14–17

No systematic study of the blue-green algae of North and South Carolina has ever been attempted. Ravenel in 1869 and 1877, Wolle in 1887, and Green in 1897 have made known the only identifications from these states. The lists submitted by these men are much too meager to give any idea of the relative abundance of the species of this class. Ravenel has identified 14 species from South Carolina, Wolle 2 species, and Green 3 species. Ravenel and Green have published 1 species each from North Carolina. These 21 species constitute the recorded blue-green flora of this region up to the present time. Unfortunately the writer has had no opportunity to examine the specimens of the above collectors.

It is interesting to note that no species of the families Chroococcaceae, Chamaesiphonaceae, or Rivulariaceae has ever been recorded.

Systematic work on the blue-green algae was begun in North Carolina in the fall of 1930. The boundaries were later extended to include South Carolina as well. Samples of blue-green algae were collected from time to time from these states until the spring of 1934. The submitted list of species is far from being complete, but it probably contains at least the more common forms.

The writer wishes to express his thanks to Miss Josephine E. Tilden, former Professor of Botany at the University of Minnesota, under whose able guidance this work was accomplished, and to Professor E. L. Green, of the Ancient Language Department at the University of South Carolina, for having supplied the Latin descriptions for new species.

METHOD

The method of collecting and preserving Cyanophyceae for future identification is simplified considerably because of the hardiness of these

* A doctoral thesis submitted to the Graduate Faculty of the University of Minnesota.

plants and because they do not plasmolize readily. A 5% solution of formaldehyde proved to be the best fixing and preserving agent. In this solution, the plants keep for an indefinite period of time.

Upon being collected in the field, the material was placed in small vials containing the fixing solution. Those intended for immediate study were brought into the laboratory in water.

For identification purposes, the material is best studied from semi-permanent mounts, preferably glycerine mounts. Glycerine not only holds the plants in a stationary position, but also causes the trichomes to become more transparent. To make these mounts a small sample of the material is taken from the formaldehyde solution and thoroughly washed in water. It is then transferred to a 10% glycerine solution and exposed to the air to permit evaporation. When the glycerine has become sufficiently concentrated, the plant mass is gently teased apart and portions are transferred onto a slide in a drop of pure glycerine. A cover glass is added and the material is ready for study. The mount is less likely to be destroyed if sealed with balsam, or some similar substance.

With unicellular forms, the washing process may be omitted as many plants are likely to be lost. The small amount of formalin remaining in the glycerine does not noticeably affect the plants.

The accompanying drawings, all of which are original, were made by the method employed in the phycological laboratories of the University of Minnesota (see Tilden in bibliography).

CLASSIFICATION

The system of classification here adopted is essentially that which has been in general use since the latter part of the nineteenth century. Minor differences will be observed, however.

Order I. COCCOGONALES. Plants unicellular, single or associated in colonies which are usually embedded in a mucous integument, never filamentous; reproduction usually by simple division of the parent cell into two daughter cells, more rarely by the formation of "endogonidia."

Family 1. CHROOCOCCACEAE. Plants unattached, showing no differentiation between base and apex; reproduction by division in one, two, or three planes.

Family 2. CHAMAESIPHONACEAE. Plants attached, showing a decided differentiation between base and apex; reproduction by the formation of "endogonidia."

Order II. HORMOGONALES. Plants multicellular and filamentous; filaments branched or unbranched, consisting, usually, of one or more rows of cells surrounded by a sheath; reproduction by means of hormogones or hypnogonidia.

Family 3. OSCILLATORIACEAE. Plants simple or branched; sheaths variable, invisible or distinct; trichomes simple, consisting of a single row of vegetative cells having a constant diameter except at the apex; heterocysts absent; reproduction by the formation of hormogones.

Family 4. PLECTONEMATACEAE. Filaments in free-floating caespitose masses or densely interwoven to form felts or mats; sheaths firm, rather thin, sometimes lamellose, hyaline or rarely yellow-brown; false branches frequent, usually in pairs; trichomes fitting closely within the sheaths, often slightly constricted between the cells; heterocysts absent; reproduction by hormogones.

Family 5. NOSTOCACEAE. Individual sheaths mostly indistinct, confluent; trichomes usually much twisted and contorted, consisting of vegetative cells, heterocysts, and often hypnogonidia; reproduction by the formation of hormogones and hypnogonidia.

Family 6. SCYTONEMATACEAE. Filaments exhibiting false branching; sheaths firm, homogeneous or variously layered; trichomes consisting of a single row of vegetative cells and heterocysts; reproduction by the formation of hormogones, rarely by hypnogonidia.

Family 7. STIGONEMATACEAE. Filaments exhibiting true branching; sheaths mostly wide, sometimes very irregular; trichomes consisting of one to several rows of cells; heterocysts usually scarce and poorly developed; reproduction by the formation of hormogones, hormocysts, and hypnogonidia.

Family 8. RIVULARIACEAE. Filaments exhibiting false branching; sheaths usually firm and distinct, occasionally indistinct; trichomes tapering from base to apex, usually ending in a long multicellular hair; heterocysts mostly basal; reproduction by the formation of hormogones and hypnogonidia.

Order COCCOGONALES Atkinson, 1905

Unicellular plants, single or in colonies, rarely arranged in filaments; vegetative reproduction by division in single cells.

Family CHROOCOCCACEAE Naegeli, 1849

Reproduction entirely by cell division (fission) in one, two, or three planes at right angles to one another; plants showing no distinction between basal and apical regions.

a. Plants solitary or associated in small, individual families or colonies, not surrounded by a common gelatinous tegument.....*Chroococcus*
aa. Plants associated in families or colonies, surrounded by a common gelatinous tegument.
b. Cells aggregated to form gelatinous colonies of no definite shape.
c. Individual sheaths distinct, thick, vesicle-like.....*Gloeocapsa*
cc. Individual sheaths not distinct.
d. Cells spherical; cell divisions in all directions..*Aphanocapsa*
dd. Cells oblong to cylindrical; cell division in one direction *Aphanothece*
bb. Cells aggregated to form gelatinous colonies of a definite, characteristic shape.
c. Cells pear-shaped or heart-shaped, terminating mucous stalks radiating from the center of the colony.....*Gomphosphaeria*
cc. Cells spherical, mucous stalks absent.....*Microcystis*

CHROOCOCCUS Naegeli, 1849

Plants spherical or hemispherical after division, with homogeneous or lamellated individual sheaths, solitary or in very small colonies; colonies free-floating or forming a stratum in damp places.

Chroococcus turgidus (Kuetz.) Naeg. Gattungen einzelliger algen, p. 46. 1849. (*Protococcus turgidus* Kuetz. Tab. phycol. **1:** 5, tab. 6. 1845.) *Fig. 12.*

The colonies were nearly spherical in shape, and were composed of from two to four cells. They measured 18.6 to 65 mic. in diameter. The sheaths were wide, lamellose, and a few were yellow in color. Cell diameters varied from 12.3 to 27, rarely 38 mic.

Hollow Rock, Durham County, N. C.; Alligator Lake, near Myrtle Beach, Horry County, S. C.

GLOEOCAPSA Kuetzing, 1843

Plant mass forming more or less expanded gelatinous crusts or strata on wet or dripping rocks or in damp places; plants spherical, each consisting of a cell inclosed in a vesicle-like, strongly thickened, homoge-

neous or stratified, colorless or colored mucilage sheath, associated in groups of from 2 to 32, equally distributed throughout the colony but always some distance from one another.

a. Sheaths thick, colorless; plants 7 to 11 mic. in diameter; cells 3 to 6 mic. in diameter ..... *G. conglomerata*
aa. Sheaths indistinct, yellowish or brownish; plants 12 mic. in diameter; cells 3 to 4.5 mic. in diameter ..... *G. sparsa*

Gloeocapsa conglomerata Kuetz. Tab. phycol. **1:** 16, pl. 20. 1845–1849. *Fig. 3.*

Several colonies studied varied from 20 to 44 mic. in diameter. The plants were from 6 to 10 mic. in diameter, with very wide sheaths. At times, many cells were aggregated within a single sheath. Cells measured from 3.4 to 4.9 mic.

Associated with other algae on damp ground in the greenhouse of Duke University, Durham, N. C.

Gloeocapsa sparsa Wood. Proc. Amer. Phil. Soc. **11:** 123. (1869) 1871.

Colonies were somewhat globose with wide, lamellose and yellowish sheaths. The cells measured 3 to 4.8 mic., and possessed finely granular contents.

Hollow Rock, Durham County, N. C. Growing on wet dripping rocks with species of *Stigonema* and *Scytonema*.

APHANOCAPSA Naegeli, 1849

Plants forming colonies of no definite shape; individual sheaths not distinct, confluent with the colonial envelope; cells spherical.

Aphanocapsa grevillei (Hassall) Rabenh. Flora europaea algarum **2:** 50. 1865. (*Coccochloris grevillei* Hassall. A history of the British freshwater algae, p. 318, pl. 78, fig. 7, a–b, and 8. 1845.) *Fig. 4.*

Colonies of this species were numerous in a mass of algae which covered the bottom of a lake to a depth of from 6 to 12 inches. The globose plant masses measured about 49 mic. in diameter, while the cells measured 3.2 to 5.8 mic.

Hollow Rock, Durham County, N. C.; Alligator Lake, near Myrtle Beach, Horry County, S. C.

APHANOTHECE Naegeli, 1849

Plants oblong to cylindrical, distributed throughout the colony in a solid mass; individual sheaths not distinct, confluent with the colonial envelope; colonies somewhat globose or without definite shape, sometimes endophytic.

a. Plant mass colorless.....*A. microscopica*
aa. Plant mass colored.
b. Plant mass dirty-green or yellowish-brown; cells 2.5–3 x 4.5–5.5 mic., thickly aggregated within the colony.....*A. conferta*
bb. Plant mass pale blue-green; cells 3–8 x 4.5–24 mic., usually scattered *A. pallida*

Aphanothece microscopica Naeg. Gattungen einzelliger algen, p. 59, pl. 1, H, fig. 1. 1849. *Fig. 1.*

The colonies were somewhat young since all were globose to oval in shape. They measured about 40 mic. in diameter. The cells were 3.8–4.4 x 3.8–8.2 mic., and were nearly spherical immediately after division.

Alligator Lake, Horry County, S. C. The very abundant colonies were floating in a mass of mostly unicellular algae that covered the bottom of the lake to a depth of from six to twelve inches.

Aphanothece conferta Richter. In Hauck and Richter, Phykotheka Universalis, no. 487. 1892.

The cells, which were thickly grouped together in a colorless expanded mass, measured 1.2–2.9 x 3–5.4 mic., a little smaller than the type for this species.

Durham, Durham County, N. C. Forming olive-green, expanded, gelatinous masses on the concrete wall of the aeration pool in refrigerating plant of the Duke University Hospital.

Aphanothece pallida (Kuetz.) Rabenh. Fl. eur. algarum **2:** 64. 1865. (*Palmella pallida* Kuetz. Phycol. germanica, p. 149. 1845.) *Fig. 11.*

Several *Aphanothece*-like cells were scattered in the gelatinous matrix of other algae. They measured 4.5–6.1 x 4.8–8.2 mic. The cells were too few to allow positive identification.

Durham, Durham County, N. C. Growing in a gelatinous plant mass in a wet corner of the Duke University greenhouse.

GOMPHOSPHAERIA Kuetzing, 1836

Plants forming solid, free-floating, spherical or ellipsoid colonies, the cells generally lying some distance from one another and forming a layer one cell in thickness toward the periphery of the colonial envelope, joined at base to branched mucilage stalks, which are formed from the remnants of the parent cell membranes; colonies embedded in a copious, hyaline, homogeneous, gelatinous envelope; individual sheaths distinct or confluent with the colonial envelope; cells wedge-shaped, pear-shaped, or heart-shaped.

Gomphosphaeria aponina Kuetz. var. **delicatula** Virieux. Rech. lac Jura. Ann. biol. lac. **8:** 1916. *Fig. 10.*

These colonies varied from 30 to 85 mic. in diameter and were provided with glistening, homogeneous, hyaline, sometimes yellowish teguments. The cells measured 3–5 x 5.5–8.9 mic. While a few of the cell measurements seem to coincide with those of the typical species, the greater number falls within the limits of the variety.

Alligator Lake, Horry County, S. C. Numerous colonies were scattered throughout an expanded mass of algae that covered the bottom of the lake to a considerable depth.

MICROCYSTIS Kuetzing, 1833

Plants forming solid, free-floating, globose, elongated, or clathrate colonies; individual sheaths confluent with the colonial envelope; cells spherical.

a. Cells without pseudovacuoles, 2–3 mic. in diameter..... *M. pulverea*
aa. Cells with pseudovacuoles, 3–7 mic. in diameter..... *M. aeruginosa*

Microcystis pulverea (Wood) Forti. In De Toni, J. B. Sylloge algarum **5:** 92. 1907. (*Pleurococcus pulvereus* Wood. Smith. Contrib. to Knowledge **19** (No. 241): 79. 1872.)

The pulverulent colonies were spherical or oval in shape and the hyaline teguments were observed only occasionally. The cells, whose contents were homogeneous, measured 1.8 to 3.2 mic. in diameter, with a few whose diameters coincided with those of variety *incerta* (Lemm.) Crow.

Alligator Lake, Horry County, S. C. In a mass of algae covering the bottom of the lake to a depth of from six to twelve inches; the whitish blue-green colonies not very abundant.

Microcystis aeruginosa Kuetz. Tab. phycol. **1:** 6, tab. 8. 1845. *Fig. 2.*

Globose and clathrate colonies, which were microscopic in size, were found among filaments of *Oedogonium* sp. The spherical cells measured 3–5.5 mic. in diameter and contained numerous pseudovacuoles.

Durham, Durham County, N. C. In a small temporary stream in Duke Forest, near Duke University.

Family CHAMAESIPHONACEAE Geitler, 1925

Plants erect, cylindrical, epiphytic; reproduction by gonidia formed at the apex of the cell by abstriction; plants showing a differentiation between base and apex.

CHAMAESIPHON A. Braun and Grunow, 1865

Plants epiphytic, unicellular, solitary or aggregated in dense clusters, ovoid, pyriform, cylindrical or much elongated, attached at base, often widening upward to free apex; sheath thin, ruptured at apex; reproduction by gonidia which are abstricted successively from the upper portion of the protoplast.

Chamaesiphon incrustans Grunow. In Rabenhorst, Fl. eur. algarum **2:** 149. 1865. *Fig. 7.*

The plants, abundant on a few filaments of *Oedogonium* sp., were 4–6.3 x 5–24 mic. in size. The cell contents were pale blue-green and homogeneous.

Durham, Durham County, N. C. Attached to filaments of *Oedogonium* sp. from a brook in the Duke Forest.

Order HORMOGONALES Atkinson, 1905

Multicellular plants, either single or in colonies, distinctly filamentous; vegetative reproduction by groups of cells, numbering two to many, termed hormogones.

Family OSCILLATORIACEAE Harvey, 1858

Filaments simple or branched, containing one or more trichomes; sheaths variable, more or less gelatinous; trichomes consisting of a simple row of cells uniform along their entire length, except for the apical cells which sometimes taper more or less; reproduction by means of hormogones.

a. Sheaths not or scarcely visible.....*Oscillatoria*
aa. Sheaths evident, very thin in some species.
b. Sheaths open at the extremities, containing only a single trichome.
c. Filaments partially agglutinated; sheaths thin, more or less confluent.....*Phormidium*
cc. Filaments free; sheaths thick, firm, cylindrical.....*Lyngbya*
bb. Sheaths usually closed at the extremities; containing two to many trichomes.
c. Prostrate sheaths mucous, homogeneous, colorless...*Microcoleus*
cc. Sheaths firm, more or less lamellose, hyaline or colored *Schizothrix*

OSCILLATORIA Vaucher, 1803

Trichomes simple, free, often forming dense tangled masses, cylindrical, smooth or constricted at joints, not moniliform, straight or bent, not spirally twisted; apices straight, bent, or hooked, more or less tapering; apical cell simple, calyptrate (provided with a thickened membrane), or capitate (exhibiting a "head").

a. Cells 2–10 mic. in diameter, longer than ½ their diameter.
b. Cells 4–10 mic. in size, constricted at joints; apical cell not capitate *O. tenuis*
bb. Cells 2–3 mic. in size, not constricted at joints; apical cell capitate *O. splendida*
aa. Cells 11–60 mic. in diameter, very short, not longer than ½ their diameter.
b. Cells 11–20 mic. in size, not capitate; cross walls granular..*O. limosa*
bb. Cells 16–60 mic. in size, somewhat capitate; cross walls not granular *O. princeps*

Oscillatoria tenuis Agardh. Algarum decades **2:** 25. 1813. *Fig. 8.*

The trichomes, in the material studied, varied in diameter from 6.1 to 7.3 mic. The cells measured from 2.9 to 4.1 mic. in length, with the transverse walls distinctly granular.

Durham, Durham County, N. C.

Oscillatoria splendida Greville. Flora Edinensis, p. 304. 1824.

The gradually tapering trichomes and the apically inflated cell are characteristic of the species.

Hollow Rock, Durham County, N. C. Intermixed with *Lyngbya martensiana* Meneghini from a small stream.

Oscillatoria limosa (A. G. Roth) Agardh. Dispositio algarum sueciae, p. 35. 1812. (*Conferva limosa* Roth. Catalecta botanica **3:** 197. 1797–1806.)

The trichomes were 12.3 to 14.3 mic. in diameter. Cell lengths varied from 1.7 to 3.5 mic. All transverse walls were granulated.

Old Fort, McDowell County, N. C. The plants were intermixed with *Anabaena laxa* (Rabenhorst) A. Braun, together forming a thin, blue-green coating on the bottom of Mill Creek four inches below the surface.

Oscillatoria princeps J. P. Vaucher. Histoire des Conferves d'eau douce, p. 190, pl. 15, fig. 2. 1803.

The floating plant masses were variable in size from two to twelve square inches, and were of a bright blue-green color. All of the measurements were within the limits 41–43 x 4.1–6.1 mic. No cells were constricted at the joints.

Lake Junaluska, Haywood County, N. C. The plants were matted together in large masses which floated during the hot part of the day, sinking to the bottom of the lake at night.

PHORMIDIUM Kuetzing, 1843

Filaments simple, agglutinated, forming a woolly or felt-like layer or rarely floating, attached at the base, with free ends torn and ragged; sheaths thin, transparent, mucous, agglutinated, partly or entirely diffluent; trichomes cylindrical, in some cases constricted at joints; apex of trichome often tapering, straight or curved, capitate in some species; apical cell sometimes calyptrate, or capitate.

a. Apex of trichome straight or curved, capitate.
  b. Apex of trichome straight ..... *P. favosum*
  bb. Apex of trichome curved ..... *P. autumnale*
aa. Apex of trichome always straight, not capitate.
  b. Trichomes tapering toward the apices.
    c. Cells 3–4.5 x 3.4–8 mic.; apical cell obtuse-conical .... *P. corium*
    cc. Cells 7.5–10.5 x 4–8 mic.; apical cell truncate ..... *P. crouani*
  bb. Trichomes not tapering toward the apices.
    c. Trichomes moniliform; cells 0.6–0.8 x 1.2–6.4 mic. *P. angustissimum*
    cc. Trichomes not moniliform; cells 4.5–12 x 4–9 mic. ..... *P. retzii*

Phormidium favosum (Bory) Gomont. Monog. des Oscillariées, etc., p. 200, pl. 5, fig. 14–15. 1893. (*Oscillaria favosa* Bory. Dict. classique hist. nat. **12:** 466, pl. 12. 1827.)

The gradually tapering, straight, capitate apex of the trichome is characteristic for the species. In the plants studied, the diameter of the cells was 4.5 mic., the minimum for this species.

Durham, Durham County, N. C. Forming a thin coating on wet rocks, west campus of Duke University.

Phormidium autumnale (Agardh) Gomont. Monog. des Oscillariées, etc., p. 207, pl. 5, fig. 23–24. 1893. (*Oscillatoria autumnale* Agardh. Dispositio algarum sueciae, p. 36. 1812.) *Fig. 9.*

The deep blue-green mats formed tufts of wide extent, a single tuft being composed of many filaments whose sheaths, for the most part, had become diffluent. The lengths of the cells were a little less than for those of the species, being 3–4.1 mic., but all other characters were in agreement with the species.

Charlotte, Mecklenburg County, N. C. Forming a thin, blue-green coating on submerged rocks in a narrow creek near the city limits.

Phormidium corium (Agardh) Gomont. Monog. des Oscillariées, etc., p. 192, pl. 5, fig. 1–2. 1893. (*Oscillatoria corium* Agardh. Dispositio algarum sueciae, p. 36. 1812.)

The material studied grew in irregular, thin, slimy, light green patches on wet clay. The cells measured 4.5–9 mic. in length. No cyanophycin granules were present, but the contents of the cells were finely granular.

Durham, Durham County, N. C. Forming close, felt-like, irregular patches on the bottom of a rain pool on the campus of Duke University.

Phormidium crouani Gomont. Monog. des Oscillariées, etc., p. 195, pl. 5, fig. 5. 1893. *Fig. 5.*

The filaments formed widely expanded, mucilaginous, irregular, blue-green masses. Cell measurements fell within the limits 7.4–8.3 x 4.1–8.2 mic. The contents of the cells were finely granular and the transverse walls very distinct.

Hollow Rock, Durham County, N. C. Growing in patches on water-soaked soil.

Phormidium angustissimum W. and G. S. West. Welwitsch's African freshwater algae. Jour. Bot., Brit. and Foreign **35:** 298. 1897.

Its minute size and the moniliform nature of the trichome are features characteristic for the species.

Forming a thin blue-green coating on the bottom of a small creek at Hollow Rock, Durham County, N. C.

Phormidium retzii (Agardh) Gomont. Monog. des Oscillariées, etc., p. 195, pl. 5, fig. 6–9. 1893. (*Oscillatoria retzii* Agardh. Dispositio algarum sueciae, p. 36. 1812.) *Fig. 6.*

The plants grew in irregular, dull green, coarse masses. Most of the sheaths were diffluent. The trichomes, which were steel blue-green in color, were long and straight, the apical cell lacking a calyptra. The cells measured 5.3–6.1 x 4.5–9 mic. Their contents were finely granular.

Durham, Durham County, N. C. Growing intermixed with *Vaucheria* sp. two inches above the water level on the wet bank of a small stream.

LYNGBYA C. A. Agardh, 1824

Filaments free or aggregated into dense, floccose, caespitose masses, simple, or very rarely branched in some species, attached or free-floating; sheaths firm, of varying thickness, thin and homogeneous or thick and lamellated, colorless or rarely yellowish-brown; trichomes not constricted at the dissepiments, tapering at the apex only slightly or not at all; wall of apical cell sometimes thickened, forming a calyptra.

a. Cells 4–6 mic. in diameter ..... *L. aerugineo-caerulea*
aa. Cells 6–28 mic. in diameter.
b. Cells 6–10 x 1.7–3.3 mic.; apical cell not capitate; sheaths colorless *L. martensiana*
bb. Cells 8–28 x 2.7–5.6 mic.; apical cell capitate; sheaths becoming yellowish or brownish ..... *L. aestuarii*

Lyngbya aerugineo-caerulea (Kuetz.) Gomont. Monog. des Oscillariées, etc., p. 166, pl. 4, fig. 1–3. 1893. (*Oscillaria aerugineo-caerulea* Kuetz. Phycol. generalis, p. 185. 1843.)

Most of the plant masses were dark blue-green and slimy to the touch. The sheaths were colorless and somewhat firm, and the cells, with granular contents, were blue-green in color. The latter measured 3.9–5.9 x 2.1–3.6 (rarely 5.4) mic.

Durham, Durham County, N. C. Forming slimy masses about one square inch in area on the bottom of a small stream.

Lyngbya martensiana Meneghini. Conspectus algologiae euganeae, p. 12. 1837. *Fig. 22.*

The plant mass was decidedly blue-green in color and composed of many filaments with thick and distinct sheaths, closely matted together. The cell measurements were 8.1–9.5 x 1.6–2.25 mic. In a few plants a thin calyptra was observed. Cross walls were marked by cyanophycin granules.

Durham, Durham County, N. C.

Lyngbya aestuarii (Mertens) Liebman. Bemerkninger og tilläg til den danske Algeflora. Kröyers Tidskrift, p. 492. 1841. (*Conferva aestuarii* Mertens. Jürg. alg. aquat. II, no. 8. 1816.) *Fig. 27.*

Two separate collections of this species were made, one in which the size of the trichomes fell just below the minimum size for the species, the other in which they were just under the maximum size. In the former, the plant mass was composed of comparatively few filaments, loosely arranged. The trichomes were provided with slightly tapering and capitate apices, with transverse walls marked by two rows of granules. In this collection, the cells measured 6.8–8.6 x 2–3.7 mic.

The plant mass in the second collection was of a much brighter blue-green color, and the filaments more compactly arranged. Here, too, the trichomes tapered at the ends and were capitate. Also the transverse walls were marked by two rows of granules. The cells measured 18.4–18.9 x 2.2–4.1 mic.

Hollow Rock, Durham County, N. C. Plants of both collections forming blue-green masses on moist earth near the water-level of a small creek.

MICROCOLEUS Desmazières, 1823

Plants living on soil, in fresh water or sometimes in salt water; filaments simple or vaguely branched, creeping on the ground, sometimes growing among other algae; sheaths colorless, more or less regularly cylindrical, not lamellose, in many species finally diffluent; trichomes many within the sheath in well developed filaments, closely crowded, often twisted into rope-like bundles; apex of trichome straight, tapering; apical cell acute, rarely obtuse conical, in one species capitate.

Microcoleus vaginatus (Vaucher) Gomont. Jour. de Bot. (Morot) **4**: 353. 1890; Monog. des Oscillariées, etc., p. 93, pl. 14, fig. 12. 1893. (*Oscillatoria vaginata* Vaucher. Histoire des Conferves d'eau douce, p. 200, pl. 15, fig. 13. 1803.) *Fig. 13.*

The plant mass was deep dark green in color. It was estimated that within a single filament there were about 80 trichomes. These had very distinct transverse walls, a few were capitate, and all were tapering at the apices. The cells varied in length from 3.1 to 4.1 mic. and displayed finely granular contents.

Durham, Durham County, N. C. Forming dark-colored irregular masses on wet soil in the greenhouse of Duke University.

SCHIZOTHRIX Kuetzing, 1843

Plants living on moist earth, dripping rocks, or in inundated places; filaments prostrate, caespitose, or forming erect or prostrate *Symploca*-like fascicles or a pannose stratum, frequently branched; sheaths thin or thick, colorless or variously colored, regular or irregular in outline, firm or diffluent, in some species encrusted with calcium carbonate and hardened; trichomes straight or spirally twisted, numerous, rarely solitary within the sheath.

a. Filaments agglutinated; trichomes evidently constricted at joints; cells 3–6 x 4–11 mic. ..... *S. friesii*
aa. Filaments not agglutinated; trichomes sometimes constricted at joints; cells 3.5 x 3.5–7 mic. ..... *S. aikenensis*

Schizothrix friesii (Agardh) Gomont. Monog. des Oscillariées, etc., p. 54, pl. 9, fig. 1–2. 1893. (*Oscillaria friesii* Agardh. Synopsis algarum Scand., p. 107. 1817.) *Fig. 19.*

The plant mass, which was bright blue-green in color, consisted of a basal prostrate portion from which tufts of filaments arose to a height of 3 or 4 centimeters. The sheaths were somewhat roughened, containing sometimes one but usually two to four trichomes which were constricted at the joints and often moniliform. All of the sheaths were thick and colorless. The cells, with coarsely granular contents, measured 3.6–5.3 x 4.1–11.4 mic.

Hollow Rock, Durham County, N. C. Forming irregular, olive-green masses on damp soil and on a moist sandstone cliff.

Schizothrix aikenensis (Wolle) n. comb. (*Hypheothrix aikenensis* Wolle. Bull. Torrey Bot. Club **6:** 182. 1877.) *Fig. 24.*

The plant mass was of a dull blue-green color, very compact and thin. It consisted of densely matted filaments whose trichomes were sometimes constricted at the joints but never moniliform. Numerous granules were present in the cells. The cells measured 3.6 x 4.3–6.1 mic.

The species was originally described by Wolle as *Hypheothrix aikenensis* from a collection made at Aiken, S. C., by Ravenel. According to more recent systems of classification, the genus *Hypheothrix*, along with the genera *Symplocastrum*, *Chromosiphon*, and *Inactis*, is included as a section of the genus *Schizothrix*. This scheme is followed in the present report.

Durham, Durham County, N. C. Plant mass living on decaying vegetable matter in a stagnant pool on the Duke University campus.

Family PLECTONEMATACEAE Tilden, 1935

Filaments in free-floating caespitose masses or densely interwoven to form felts or mats; sheaths firm, rather thin, sometimes lamellose, hyaline or rarely yellow-brown; false branches frequent, usually in pairs; trichomes fitting closely within the sheaths, often slightly constricted between the cells; heterocysts absent; reproduction by hormogones.

PLECTONEMA Thuret, 1875

Plants growing among damp mosses, on wet rocks, or floating free; sheaths present; trichomes frequently constricted at the joints, exhibiting false lateral branching caused by rapid growth in certain portions, resulting in a rupture of the sheath; branches single or in pairs; apices straight, rarely tapering, without a calyptra.

Plectonema tenue G. Thuret. Ann. Sci. Nat. Bot., ser. 6, **1**: 380. 1875.

The more or less expanded plant mass was of a yellowish-brown color, due to the pigments in the sheaths. A sheath usually enclosed only a single trichome, but frequently two trichomes were observed. These gradually tapered at the apices. The cells measured 5.2–10.9 x 3.4–7.3 (rarely 8.1) mic. Terminating each trichome was a conical apical cell. The cell contents were either homogeneous or granular, and blue-green in color.

Marietta, Greenville County, S. C.

Family NOSTOCACEAE Naegeli, 1847

Colonies floating, single or gregarious, of indefinite, globose, ovoid or regularly lobate form or irregularly expanded, solid or hollow, soft or firmly gelatinous; sheaths more or less distinct, often not present, mostly confluent; trichomes unbranched, never tapering to a hair, the extremities usually similar; heterocysts present; reproduction by hormogones and hypnogonidia.

a. Heterocysts and hypnogonidia intercalary.
  b. Plant mass of indefinite shape ..... *Anabaena*
  bb. Plant mass of definite shape ..... *Nostoc*
aa. Heterocysts terminal and hypnogonidia contiguous to them *Cylindrospermum*

ANABAENA Bory, 1822

Filaments, or trichomes, almost always aquatic, solitary, free-floating, or united loosely by the mucous teguments into a mucous stratum, never

approaching in consistency the tough thallus of *Nostoc;* sheaths usually lacking, in some species delicate or copious, gelatinous, hyaline, homogeneous, rarely confluent with the mucous layer; trichomes cylindrical or slightly tapering at the ends and with a conical apical cell, usually rigid and fragile, very rarely contorted, straight, spiral, or circinate, solitary or interwoven to form flocculent masses of small size; cells spherical to barrel-shaped, rarely cylindrical, never disk-shaped; heterocysts approximately spherical, always intercalary, numerous, solitary; reproduction by hormogones and hypnogonidia; hypnogonidia solitary or in short catenate series, spherical to cylindrical, generally much more elongated than those of *Nostoc*, either adjacent to the heterocysts or developed between them.

a. Plants living in the tissues of other plants.....*A. cycadeae*
aa. Plants not living in the tissues of other plants.
b. Trichomes circinate; hypnogonidia cylindrical, curved..*A. flos-aquae*
bb. Trichomes straight or flexuous; hypnogonidia spherical, ovate or cylindrical, not curved.
c. Hypnogonidia spherical, spherical-compressed, or ovoid *A. parva*
cc. Hypnogonidia cylindrical.
d. Hypnogonidia usually remote from the heterocysts, or contiguous to them on one side.....*A. laxa*
dd. Hypnogonidia always contiguous to and developing on both sides of the heterocysts.....*A. torulosa*

Anabaena cycadeae J. Reinke. Bot. Zeit. **37:** 473, pl. 6, fig. 1–5. 1879.

The habit of growth of this species apparently necessitates the formation of trichomes composed of only a few cells. The spherical to barrel-shaped cells varied considerably in size, measuring 2.7–6.3 x 2.7–7.2 mic. Most of the heterocysts were spherical, measuring 5.4–6.3 x 5.4–6.3 mic. in size.

Durham, Durham County, North Carolina. Causing the formation of long, branched nodules on the roots of *Zamia florida* in the greenhouse of Duke University.

Anabaena flos-aquae (?) (Lyngbye) Brebisson. In Brebisson and Codey, Algae des environs de Falaise, p. 36. 1835. (*Nostoc flos-aquae* Lyngbye. Tentamen hydrophytologiae danicae, p. 201, table 68. 1819.)

The absence of gonidia in the materials studied makes this identification uncertain. The presence of pseudovacuoles indicates the plant to be a water-bloom species. Because all available measurements agree

with the corresponding descriptions for this species, the writer places the few scattered plants that were observed in this species. The spherical compressed vegetative cells measured 4.5–5.9 mic. in diameter, and the heterocysts were slightly larger.

Durham, Durham County, N. C. Intermixed with other algae growing in a small creek in the Duke Forest.

Anabaena parva sp. nov. *Fig. 25.*

Plantae massa glutinosa, expansa in solo humido aut libere fluitante, caerulei-viridi; vaginis invisibilibus; trichomatibus modice rectis, constrictis ad articulos; cellis ovatis vel ellipticis in forma; heterocystis sphaericis vel ellipticis; gonidiis sphaericis vel ellipticis in forma, numerosis in serie catenata, remotis ab heterocystis, evolutis centrifuge, continentibus multa et magna granula cyanophycina; contento cellae paris generis, caerulei-viridi.

Cellis 2.9–4.1 x 4.1–8.2 mic.
Heterocystis 4.1–5.3 x 4.5–8.2 mic.
Gonidiis 4.5–7.4 x 3.6–8.2 mic.

Plant mass gelatinous, expanded, spreading on damp soil or free-floating, blue-green; sheaths invisible; trichomes moderately straight, constricted at the joints; cells oval or elliptical in shape; heterocysts spherical or elliptical; gonidia spherical or elliptical in shape, numerous in catenate series, remote from the heterocysts, developing centrifugally, containing numerous large cyanophycin granules; cell contents homogeneous, blue-green.

Cells 2.9–4.1 x 4.1–8.2 mic.
Heterocysts 4.1–5.3 x 4.5–8.2 mic.
Gonidia 4.5–7.4 x 3.6–8.2 mic.

Anabaena variabilis Kuetzing is the species most closely indicated by the appearance of the plants of this collection. There can be no confusion, however, after careful study. The plants of this new species are much smaller in every respect.

Myrtle Beach, Horry County, S. C. Forming large, irregular gelatinous masses floating on the surface of Lake Chapin. Collection:—S. C. 42. July 4, 1933.

Anabaena laxa (Rabenh.) A. Braun. Bull. Soc. Bot. Fr. **32:** 120, pl. 4, fig. 2–3. 1885. (*Sphaerozyga laxa* Rabenh. Fl. eur. algarum **2:** 193. 1865.) *Fig. 34.*

The trichomes of this collection were quite straight. Heterocysts, which were usually elliptical in shape, measured 4.5–6.3 x 6.1–9 mic.

The spherical or short-cylindrical cells were 4.1–4.5 x 3.2–4.1 mic. The distinguishing feature of this species, and one that separates it from *Anabaena inequalis* (Kuetz.) Bornet and Flahault, is the colorless wall of the gonidium. The hypnogonidia were cylindrical and measured 5.4–6.3 x 12.6–20.5 mic.

Old Fort, McDowell County, N. C. Forming a thin, blue-green coating on mud in the bottom of a small creek.

Anabaena torulosa (Carmichael) Lagerh. Oefversigt af Kengl. Vetensk. Akad. Förhandl. **40:** 47. 1883. (*Belonia torulosa* Carmichael. In Hooker, British Flora **2:** 379. 1833.) *Fig. 33.*

The vegetative cells that were measured for this determination fell within the limits of 3.9–4.8 x 2.6–3.6 mic. and were spherical or barrel-shaped. All of the heterocysts were nearly spherical and measured 5.4–7.2 mic. in diameter. Adjacent to the heterocysts were long, cylindrical gonidia, 7.2–9 x 10.8–19.8 mic. They developed centripetally.

Hollow Rock, Durham County, N. C. Forming gelatinous masses near the edge of a small stream.

NOSTOC Vaucher, 1803

Colonies solid or hollow, mucous, gelatinous, or coriaceous, composed of a more or less firm jelly with a denser limiting layer, usually globose or oblong when young, later either remaining so or assuming a variety of forms in the various species, frequently breaking open to form extensive flattened expansions, often with lacerated margins, terrestrial or aquatic, attached to a substratum or free-floating; filaments flexuous, densely intertwined and contorted, generally more closely crowded toward the exterior; sheaths often not present or inconspicuous, sometimes distinct in portions of the colony, especially near the periphery, colorless or not uncommonly yellow or brown, firm, thick, mucous or gelatinous, mostly confluent, the gelatinous colony being made up of dissolved individual sheaths; trichomes torulose or moniliform, consisting of a single row of uniform cells, unbranched, showing no differentiation of base and apex; vegetative cells spherical, barrel-shaped or cylindrical; heterocysts larger than, but generally the same shape as, the vegetative cells, spherical to oblong, terminal or intercalary, often in series; reproduction by means of hormogones and hypnogonidia; gonidia spherical to oblong, intercalary, seriate, beginning midway between the heterocysts and developing centrifugally.

a. Colonies microscopic.
  b. Filaments loosely flexuous; sheaths wide, bulbous; trichomes distinct ..... *N. paludosus*
  bb. Filaments densely entangled; sheaths close, transparent; trichomes scarcely distinct ..... *N. punctiforme*
aa. Colonies not microscopic.
  b. Cells 2.3–3.6 x 10.8–19.8 mic., cylindrical; hypnogonidia oval, elliptical, or cylindrical, frequently curved ..... *N. ellipsosporum* var. *minimum*
  bb. Cells 3–4 x 7–8.6 mic., barrel-shaped or cylindrical; hypnogonidia oblong, never curved ..... *N. muscorum*

Nostoc paludosum Kuetz. Tab. phycol. **2:** 1, pl. 1, fig. 2. 1850.

The colonies, somewhat microscopic, are larger than those of *Nostoc punctiforme* (Kuetzing) Hariot. The trichomes were provided with very evident inflated sheaths. Measurements of the cells fell within the limits of 3.2–3.6 x 3.2–5.6 mic. The spherical-shaped heterocysts measured 4.2 mic. in diameter. Gonidia were not observed. Granules were abundant within the cells.

Hollow Rock, Durham County, N. C. Embedded in a gelatinous mass formed by other algae. On the wet bank of a small stream.

Nostoc punctiforme (Kuetz.) Hariot. Jour. de Bot. (Morot) **5:** 30. 1891. (*Polycoccus punctiforme* Kuetz. Phycol. generalis, p. 171. 1843.) *Fig. 23.*

The colonies, always microscopic in size, were spherical or somewhat flattened in shape, and contained, usually, only a few much twisted and contorted trichomes. In form, the cells were quite variable, being, usually spherical or spherical-compressed. They measured 3.2–4 x 3.2–4 mic. The heterocysts were few in number, and small. No gonidia were observed.

Hollow Rock, near Durham, Durham County, North Carolina. Forming, with other unicellular species of blue-green algae, a thin coating on submerged rock in a nearby stream.

Nostoc ellipsosporum (Desmaz.) Rabenh. var. **minimum** var. nov. *Fig. 21.*

The plants under consideration differ from *Nostoc ellipsosporum* (Desmaz.) Rabenhorst only in the size of the vegetative cells, which are much smaller and longer than in the species. In the species the cells measure 4 x 6–14 mic., while in this new variety they measure 2.4–3.6 x 10.8–24 mic. The heterocysts and gonidia conform to those of the

variety both in size and shape, with the exception that the former appear to be slightly broader in the species.

Alligator Lake, near Myrtle Beach, Horry County, South Carolina. Forming blue-green or brown gelatinous masses among mosses in damp places on the bank of the lake.

Nostoc muscorum (?) Agardh. Dispositio algarum sueciae, p. 44. 1812.

Gonidia were not present in this collection and thus the writer cannot be certain of this identification. He is, however, reasonably satisfied. The much twisted and contorted trichomes measured 3.5–4.5 mic. in diameter, and were composed of barrel-shaped cells 7–8.6 mic. in length. In size, the globose heterocysts varied between the limits 5.2–6.8 mic. in diameter. Cell contents were quite granular.

Durham, Durham County, North Carolina. Forming expanded gelatinous masses on moist soil and among mosses. Collected in the Duke Forest.

CYLINDROSPERMUM Kuetzing, 1843

Sheaths diffluent, forming an amorphous, expanded mucous stratum of indefinite form; trichomes relatively short because of frequent hormogone formation, sometimes exhibiting movement; vegetative cells cylindrical, longer than broad; heterocysts cylindrical, longer than broad, solitary, always terminal; hypnogonidia always developed from the cell or cells next to the heterocyst at base of plant, generally solitary, rarely in series, outer layer of wall papillate in some species.

a. Wall of gonidium smooth.
  b. Hypnogonidia 9–21 x 20–30 mic., with radial striations in outer wall ..... *C. alatosporum*
  bb. Hypnogonidia 9–12 x 10–20 mic., without such striations ..... *C. muscicola*
aa. Wall of gonidium covered with numerous short needle-like spines ..... *C. trichotospermum*

Cylindrospermum alatosporum F. E. Fritsch. Ann. of the South African Mus. **9:** 578, fig. 37, d-h. 1911–1918. *Fig. 20.*

Vegetative cells studied from this collection measured 3.6 x 3.6–4.8 mic.; heterocysts were 4.5–5.1 x 6.2–7.5 mic.; hypnogonidia were 7–12 x 16.6–22 mic. It will be noticed that a few of these measurements are a little smaller than those of the typical species, but not to such an extent as to affect the ultimate identification. Most of the vegetative cells

were barrel-shaped. The ellipsoid gonidia developed singly and possessed rounded instead of truncate apices. The peculiar and characteristic striations of the walls of the gonidia were well in evidence.

Durham, Durham County, N. C. Associated with *Vaucheria* sp. in freshwater in a laboratory at Duke University.

Cylindrospermum muscicola Kuetz. Phycol. germanica, p. 173. 1845. *Fig. 18.*

The slimy plant mass was dark green in color. Vegetative cells measured 3.2–3.6 x 2.5–4.1 mic.; the heterocysts 5.4–6.3 x 5.4–7.2 mic.; the gonidia 8.1–8.2 x 16.4–19.8 mic. The cells were somewhat quadrate in shape and constricted at the joints. The measurements of the heterocysts were slightly larger than those for the species, but this was not of sufficient importance to alter the identification.

Hollow Rock, Durham County, N. C. Forming a thin coating on moist clay on the bank of a small stream.

Cylindrospermum trichotospermum Frémy. Les Myxophycées de l'Afrique équatoriale francaise, p. 379, fig. 316. 1930. *Fig. 26.*

This species is characterized by the elliptical gonidia, the walls of which are provided with numerous needle-like spines. The vegetative cells from this collection measured 3.2–3.6 x 4.5–7.2 mic.; the heterocysts 4.5–5.5 x 8.1–9 mic.; the gonidia 11.8–14.1 x 19.8–28.8 mic.

In the original description of this species the plant mass is said to be "minute, floating among other algae." The North Carolina material, however, occurred on mud forming plant masses about the size of a silver dollar. These masses were composed of only a single species.

Old Fort, McDowell County, N. C. Plant masses, formed on mud, shaded by tall sedges.

Family SCYTONEMATACEAE (Kuetzing) Rabenhorst, 1865

Filaments differentiated into a basal and terminal portion, simple or branched; false branches formed by the perforation of the sheath by the trichome, which thereupon issues as one or two long flexuous branches, each developing a sheath of its own; sheaths homogeneous and colorless, or lamellose and yellowish or brownish, firm and tubular; trichomes consisting of a single row of cells, one or more included within the sheath; heterocysts and hypnogonidia variously disposed; reproduction by means of hormogones and hypnogonidia.

a. False branches usually arising between two heterocysts, single or in pairs; sheaths delicate or very thick, homogeneous or lamellose; lamellae parallel, or more or less diverging toward the apex.....*Scytonema*
aa. False branches usually arising in the immediate region of the heterocysts, single; sheaths somewhat thin, flexuous, more or less fragile..*Tolypothrix*

SCYTONEMA C. A. Agardh, 1824

Plants generally forming a dense interwoven mass on damp or wet substrata, more rarely submerged; filaments branched, containing a single trichome; sheaths tubular, of practically uniform thickness, delicate or thick, firm, homogeneous and colorless, or lamellose, with parallel or more or less diverging layers, their edges in the latter case sometimes projecting and producing a frayed margin, termed ocrea, yellowish or brown in color; false branches long and flexuous, developing a sheath of their own, arising singly or more commonly in pairs between two heterocysts, formed by the lateral perforation of the sheath by the trichomes at first pushing out as a loop, which later breaks across the apex; trichomes straight, cylindrical, toward the growing end of the filament, often increasing in diameter, the cells becoming shorter and rounded; heterocysts intercalary; reproduction normally by hormogones, and by hypnogonidia in many species; hormogones formed at the ends of branches; hypnogonidia spherical or oval, with a thin, smooth wall.

a. Sheaths homogeneous or lamellose, not with diverging layers.
b. Plants aquatic.....*S. crispum*
bb. Plants aerial or subaerial.
c. Sheaths homogeneous; trichomes increasing in diameter toward the apices; cells 5–12.8 mic. in diameter.....*S. carolinianum*
cc. Sheaths lamellose; trichomes more or less constant in diameter throughout; cells 25–33 mic. in diameter.....*S. insigne*
aa. Sheaths lamellose, with diverging layers.....*S. crustaceum*

Scytonema crispum (Agardh) Bornet. Bull. Soc. Bot. Fr. **36:** 156. 1889. (*Oscillatoria crispum* Agardh. Synopsis algarum Scand., p. 108. 1817.)

The plant mass was woolly in appearance and greenish blue-green in color. All of the filaments measured between 18 and 31.5 mic. in diameter, with sheaths sometimes lamellose. False branches were always in pairs, and most of the trichomes were constricted at the joints. The numerous heterocysts were slightly broader than the vegetative cells and depressed-spherical to quadrate in shape. The cells measured 15–29 x 5–10.5 mic. in size, with slightly granular contents and a bright blue-green color.

Lake Chapin, near Myrtle Beach, Horry County, S. C. Forming large woolly masses attached to *Chara* sp. and other plants growing in the lake.

Scytonema carolinianum sp. nov. *Fig. 15–16.*

Filamentis cum aliis algis mixtis, assequentibus diametrum maximum ad apices et decrescentibus ad mediam regionem; falsis ramis longis, plerumque in paribus, agglutinatis aliquod spatium a base; vaginis sine colore, latis, mucosis, homogeneosis; trichomatibus 5 mic. in diametro prope mediam regionem, crescentibus ad 12.8 mic. ad apices; minima longitudine cellarum attacta in meristema parte ad fines trichomatum; heterocystis sphaericis, vel brevi-cylindratis; contento cellarum granulato, viridi usque ad caerulei-viridem in colore.

Filamentis 10.8–28.5 mic. in diametro.

Cellis 5–12.8 x 2.4–14.8 mic.

Heterocystis 8.3–10.5 x 8.3–12.2 mic.

Filaments intermixed with other algae, reaching their maximum diameter at the apices and decreasing toward the mid-region; false branches long, usually in pairs, agglutinated for some distance from the base; sheaths colorless, wide, mucous, homogeneous; trichomes 5 mic. in diameter near the mid-region, increasing to 12.8 mic. at their apices; minimum length of cells reached in the meristem portion at the ends of the trichomes; heterocysts spherical, or short cylindrical; cell contents granular, green to blue-green in color.

Filaments 10.8–28.5 mic. in diameter.

Cells 5–12.8 x 2.4–14.8 mic.

Heterocysts 8.3–10.5 x 8.3–12.2 mic.

This species appears to be characterized by the gradual diminution in diameter of the trichomes from the apices to the mid-region. The plants were abundant in a single collection.

Hollow Rock, Durham County, N. C. Filaments intermixed with those of *Stigonema hormoides* (Kuetz.) Bornet and Flahault. Growing on damp soil rich in decaying vegetable matter. Collection:—5a.

Scytonema insigne W. and G. S. West. Welwitsch's African freshwater algae. Jour. Bot., Brit. and Foreign **35:** 266. 1897.

This plant proved to be very interesting, not having been collected since W. and G. S. West first described it from Africa in 1897. The plant mass, greenish in color, was somewhat widely expanded. The filaments, measuring 18–26.5 mic. in diameter, formed erect tufts.

Each filament was provided with a thick, lamellose sheath formed of parallel layers. The yellowish color of the inner layers of the sheath, observed here and there, was accentuated by the colorless outer ones. The cells measured 8.2–12.3 x 3.6–16.4 mic. and appeared constricted at the joints.

Hollow Rock, Durham County, N. C. Growing in patches on wet soil and on damp limestone rocks.

Scytonema crustaceum Agardh. Systema algarum, p. 39. 1824. *Fig. 17.*

The plant mass was brown in color, widely expanded, and calcareous but somewhat velvety to the touch. It consisted of filaments, 16–28.8 mic. in diameter, whose false branches occurred in pairs at the base of the mass, but only singly in the erect branches. All of the sheaths were firm and thick, with decidedly diverging layers, and very irregular in outline. The cells were somewhat spherical-depressed in shape, usually shorter than broad, and constricted at the joints. Most of the heterocysts were spherical to spherical-compressed. Cell contents appeared to be either homogeneous or with a few large granules.

Myrtle Beach, Horry County, S. C. Forming widely expanded, but relatively thin, brown patches on dry ground near Lake Chapin.

TOLYPOTHRIX Kuetzing, 1843

Plants chiefly aquatic, occurring among various plants in ponds and lakes, forming floccose floating masses or submerged tufts, or, rarely, a stratum on damp rocks; filaments branched; sheaths rather thinner than those of *Scytonema*, colorless or yellowish or brown, either flexible or more or less fragile; false branches more or less frequent, usually single and mostly issuing from the main filament in the immediate region of the heterocysts, very rarely between two heterocysts; heterocysts single or from 2 to 5 in a series; reproduction normally by means of hormogones, sometimes by hypnogonidia; hormogones formed at the ends of the branches; hypnogonidia spherical, oval or ellipsoid, often many in a series, with smooth, thin walls.

a. Filaments under 5 mic. in diameter ..... *T. delicatula*
aa. Filaments over 5 mic. in diameter.
b. Plants aquatic ..... *T. distorta*
bb. Plants aerial, living on trunks of trees ..... *T. rechingeri*

Tolypothrix delicatula sp. nov. *Fig. 28.*

Plantae massa lanata, spinulari forma, colore caerulei-viridi, filamentis rare scissis, ramis falsis brevibus et patulis; vaginis maxime

astrictis, indistinctis nisi ad fines trichomatum, sine colore; cellis non omnino vel vix constrictis ad articulos; heterocystis, prope ramos, sphaericis usque ad oblongos, qui sunt remoti a ramis cadiformibus usque ad cylindratos; partibus interioribus caerulei-viridibus, habentibus granula sparsa.

Filamentis 4.1–4.5 mic. in diametro.
Cellis 4.1–4.5 x 12.3–18.4 mic.
Heterocystis 4.1–6.9 x 5.3–31.5 mic.

Plant mass woolly, cushion-shaped, blue-green in color; filaments rarely branched; false branches short, spreading; sheaths very close, indistinguishable except at the ends of the trichomes, colorless; cells not at all or scarcely constricted at the joints; heterocysts, adjacent to the branches, spherical to oblong, those remote from the branches barrel-shaped to cylindrical; cell contents blue-green, containing scattered granules.

Filaments 4.1–4.5 mic. in diameter.
Cells 4.1–4.5 x 12.3–18.4 mic.
Heterocysts 4.1–6.9 x 5.3–31.5 mic.

This material was collected in abundance and studied in both the living and in preserved conditions. At first glance, the appearance of the plant is similar to that of *Tolypothrix tenuis* Kuetzing, but it differs from this species in several characteristics. In the plants described above the sheath is so thin that it is invisible except at the ends of the trichomes, while in *T. tenuis* the sheath is seen distinctly in any portion of the plant.

The cells of *T. tenuis* rarely fall below 5 mic. in diameter and they are as long as broad or sometimes longer than broad. The heterocysts are spherical. In the case of the plants of the new species, on the other hand, the cells never exceed 4.5 mic. in diameter and are 12.3 to 18.3 mic. in length, making them cylindrical in shape. The heterocysts are spherical, barrel-shaped, or cylindrical.

Alligator Lake, Horry County, S. C. Forming slimy, irregularly expanded, submerged masses on an unidentified species of *Chara*. Collection:—S. C. 3. July 2, 1932.

Tolypothrix distorta (Flora Danica) Kuetz. Phycol. generalis, p. 228. 1843. Flora Danica, pl. 820. 1780.

The plant mass was more or less expanded, blue-green in color, and consisted of filaments which varied in diameter from 11.2 to 14.1 mic. False branches were very abundant, and long and graceful in appearance. The trichomes, which possessed thin and colorless sheaths, were 8.2 to 10.1 mic. in diameter, with a few below the minimum for the

species. Cells of this species, many of which were constricted at the joints, measured 2.5 to 9.4 mic. in length. Their contents appeared homogeneous and blue-green in color.

Greenville, Greenville County, S. C. Forming a more or less expanded, blue-green coating on the bottom of a shallow pool.

Tolypothrix rechingeri (Wille) Geitler. In Pascher, Die Süsswasser-Flora Deutschlands, etc., **12:** 259, text fig. 309. 1925. (*Hassallia rechingeri* Wille. Süsswasseralgen von den Samoainseln, etc. Botanische und Zoologische ergebnisse einer wissenschaftlichen forschungsreise nach den Samoainseln von März bis Dezember 1905, p. 10, pl. 1, fig. 19–26. 1914.) *Fig. 31.*

The plants collected agree in every way with the species description except that some of the trichomes were as small as 6.1 mic. in diameter. Wille in his original description states that the trichomes branch between the heterocysts, but this does not seem always to be the case. The plant mass was brown in color. The filaments measured 11 to 15 mic. in diameter, were richly branched, and were provided with yellow, very fragile sheaths. Usually, the heterocysts were spherical in shape, sometimes spherical-depressed.

Durham, Durham County, N. C. Growing in loose masses on the trunk of a cedar tree near Duke University.

Family STIGONEMATACEAE Kirchner, 1898

Plants (filaments) free, or forming a stratum or gelatinous colony, composed of a single series of cells, or of two or more series, within a sheath, resulting from cell division in one, two, or three planes, branched; true branches present; cells somewhat spherical, cylindrical, or irregularly angular; heterocysts intercalary or terminal on short lateral branches; reproduction by hormogones and by hypnogonidia.

a. Trichomes consisting of a single row of cells; branches usually unilateral ..... *Hapalosiphon*

aa. Trichomes consisting of one to several rows of cells; branches scattered, usually not unilateral ..... *Stigonema*

HAPALOSIPHON Naegeli, 1849

Plant mass caespitose-floccose, thin, usually aquatic; filaments free, in floating patches or epiphytic on higher plants, branched, consisting typically of a single row of cells (rarely of two rows) within the sheath; branches erect, usually about the same diameter as the creeping primary

filament, commonly unilateral, long, flexuous, very slightly tapering; sheaths firm, narrow, of uniform thickness; hypnogonidia with thick, yellowish-brown walls, often formed in abundance.

a. Filaments 5.5–7.5 mic. in diameter; branches short, equal to, sometimes slightly thinner than, the main filament.....*H. welwitschii*
aa. Filaments 7.2–9.5 mic. in diameter; branches long, flexuous, always thinner than the main filament.....*H. hibernicus*

Hapalosiphon welwitschii W. and G. S. West. Welwitsch's African freshwater algae. Jour. Bot., Brit. and Foreign **35:** 242. 1897. *Fig. 29.*

The main filaments observed measured 6.4 to 6.6 mic. in diameter. They occurred singly. Numerous unilateral branches were present, all of which were slightly smaller than those of the parent filaments. The cells were quite variable in size, those in the branches being longer than those in the main filament.

Hollow Rock, Durham County, N. C. Intermixed with an unidentified species of *Schizothrix*, growing near the edge of a small creek.

Hapalosiphon hibernicus (?) W. and G. S. West. Jour. Royal Micros. Soc., 1896, p. 163.

Sufficient material was not at hand to insure a positive identification. The filaments occurred singly, and measured 8.2–9.4 mic. in diameter. Cells within the main filaments fell within the limits 5.3–7.1 x 4.1–10.2 mic. The long cylindrical heterocysts were 5.6–6.2 x 18.4–20.5 mic. in size. Because these measurements are fairly suitable for the corresponding measurements for the species, the writer, with hesitancy, places the few plants in the collection in this species.

STIGONEMA C. A. Agardh, 1824

Plants, or filaments, free, terrestrial or aquatic, free-floating or aggregated to form rigid or soft felt-like or cushion-like dark brown masses on damp surfaces, frequently very wide, irregular, and often richly provided with short, thick, irregularly disposed branches that are often considerably narrower than the main filaments; sheaths extremely thick, lamellose, generally golden-yellow or brown, made up of dissolved sheaths of the contained cells, which as they grow continue to excrete gelatin; trichomes consisting of rounded cells exhibiting obvious protoplasmic connections, usually with distinct individual sheaths disposed in one, two, or three to several rows; heterocysts commonly lateral,

here and there intercalary; reproduction normally by hormogones developed in the ends of ordinary branches or in short special branches, few- or many-celled.

a. Trichomes consisting of a single series of cells.
  b. Filaments 7–15 mic. in diameter; sheaths usually colorless *S. hormoides*
  bb. Filaments 24–26 mic. in diameter; sheaths thick, lamellose, yellowish or brownish..... *S. panniforme*
aa. Trichomes consisting of two to several series of cells.
  b. Filaments 27–37 mic. in diameter; hormogones 12 x 45 mic. in size *S. turfaceum*
  bb. Filaments 40–70 mic. in diameter; hormogones 18 x 45 mic. in size *S. informe*

Stigonema hormoides (Kuetz.) Bornet and Flahault. Ann. Sci. Nat. Bot., ser. 7, **5:** 68. 1887. (*Scytonema hormoides* Kuetz. Phycol. generalis, p. 215. 1843.) *Fig. 32.*

Three features characterize this species: the comparatively small size of the filament, the colorless sheath, and the uniseriate axis. The filaments studied from this collection measured 8–12 mic. in diameter. Most of the vegetative cells were spherical and were 4.1–4.9 mic. in diameter.

Hollow Rock, Durham County, N. C. Growing in loose mats on damp soil well shaded by forest trees.

Stigonema panniforme (C. A. Agardh) Kirchner. Kryptogamen-Flora von Schlesien **2:** 230. 1878. (*Scytonema panniforme* Agardh. Synop. algarum, p. 116. 1817.)

This species is characterized by the definite size of the filaments, which contain each a single row of cells, and by the thick, yellow lamellose sheaths. The filaments in this collection measured 24.6–28.7 mic. in diameter. The hormogones were 102.5 mic. in length and contained 15 to 22 cells each. Most of the branches tapered toward the apices. The cells, frequently discoid in shape, measured 8.2–12.3 x 4.1–14.5 mic.

Hollow Rock, Durham County, N. C. Growing on moist soil near a small creek and on the surface of decaying vegetable matter.

Stigonema turfaceum (Berk.) Cooke. British freshwater algae, p. 273. 1884. (*Scytonema turfaceum* Berk. English Botany, pl. 2826, fig. 1. 1838.) *Fig. 14.*

Filaments from this collection measured 28.7–36.9 mic. in diameter. This species is terrestrial, the trichomes of which are composed of several rows of cells. The latter measured 5–12.5 x 3.5–13 mic. Hormogones were quite variable in size, some of them falling short for the minimum of the species. Each consisted of from 7 to 13 cells.

Hollow Rock, Durham County, N. C. Growing on moist earth in rocky places, associated with *Stigonema hormoides*.

Stigonema informe Kuetz. Species algarum, p. 319. 1849.

When collected, the widely expanded plant mass was dark brown in color. All of the filaments were large, coarse, and very irregular in outline, each containing several rows of cells. The filaments were 32.8–59.3 mic., the cells 11.2–17.2 mic. in diameter. Hormogones were numerous, measuring 30 to 50 mic. in length.

Hollow Rock, Durham County, N. C. Forming expanded masses on damp, well shaded soil near the bank of a small stream.

Family RIVULARIACEAE Rabenhorst, 1865

Filaments cohering by their gelatinous sheaths, forming more or less definite spherical or hemispherical colonies, radiating from the center outward, or parallel and forming caespitose strata, rarely solitary, simple or branched; trichomes tapering from base to apex, ending in a colorless hair, one to several within a sheath; branches false, usually beneath the heterocysts, more rarely between the heterocysts; heterocysts basal or intercalary, rarely absent; reproduction by means of hormogones and hypnogonidia.

a. Filaments not agglutinated, rarely branched; hypnogonidia usually not present
Calothrix

aa. Filaments agglutinated, widely branched; hypnogonidia present
Gloeotrichia

CALOTHRIX C. A. Agardh, 1824

Plants sometimes solitary, more usually forming penicillate tufts or soft velvety expansions, which are generally attached to submerged rocks and stones, some species being epiphytic; filaments simple or with scanty false branches; sheaths cylindrical, firm, frequently thick and lamellose, often diffluent in the upper portion, sometimes brown in color; trichomes tapering from near the base upward, or only at the upper end, sometimes long and flexuous, terminating in a point or fre-

quently in a long hyaline hair; heterocysts basal or intercalary, single or several in a series, absent in a few species; hormogones often formed in series; hypnogonidia formed singly or in a series adjacent to the basal heterocyst.

a. Filaments epiphytic, isolated or in small groups.....*C. genuflexa*
aa. Filaments not epiphytic, forming more or less expanded masses, sometimes free-floating; sheaths colorless.....*C. braunii*

Calothrix genuflexa sp. nov. *Fig. 30.*

Filamentis usque ad 350 mic. in longitudine, seiunctis vel parvis globis, epiphyticis; parte infirma filamenti flecta et hospiti affixa, apice libera et recta; vaginis densis, firmis, sine colore, tubulata; trichomatibus constrictis ad articulos, valde paulatim fastigalis, non in pilum finitis; cellis cadiformibus, brevioribus in regione basis; heterocystis singulis, basalibus, hemisphaericis, sine colore; rebus interioribus cellarum paululum granulatis, caeruli-viridibus.

Filamentis 9.6–12.8 mic. in diametro.

Cellis 8.2–12.7 x 7.2–11.5 mic.

Heterocystis 6.4–11.5 mic. in diametro.

Filaments up to 350 mic. in length, isolated or in small groups, epiphytic; basal portion of filament bent, attached to the host, apical portion free and erect; sheaths thick, firm, colorless, tubular; trichomes constricted at the joints, very gradually tapering, not ending in a hair; cells barrel-shaped, shorter in the basal region; heterocysts single, basal, hemispherical, colorless; cell contents somewhat granular, blue-green.

Filaments 9.6–12.8 mic. in diameter.

Cells 8.2–12.7 x 7.2–11.5 mic.

Heterocysts 6.4–11.5 mic. in diameter.

The new plant compares unfavorably with any species of *Calothrix* yet described. *Calothrix epiphytica* West and West and *C. scytonemicola* Tilden make the nearest approach. When compared with the former, the lengths of the filaments, their habit of growth, and the position of the heterocysts are identical with those of the new species. On the other hand, the diameters of the trichomes and the filaments of the proposed new species are twice those of *C. epiphytica*, and the trichome does not end in a hair, as is the case with the latter.

The plants differ from those of *C. scytonemicola* in having a much broader filament, and heterocysts not in pairs. The sheath is firm and thick, while in *C. scytonemicola* it is indistinct.

Florence, Florence County, S. C. Epiphytic on filaments of *Scytonema crispum* Ag. that were attached to a log floating in Muldrow's Mill Pond. Collection:—S. C. 31. July 3, 1933.

Calothrix (?) **braunii** Bornet and Flahault. Ann. Sci. Nat. Bot., ser. 7, **3:** 368. 1886.

The free-floating filaments were not present in sufficient numbers to allow a satisfactory identification. They measured 6–10.3 mic. in diameter, the smaller sizes referring to young filaments. The sheaths were thin, colorless, and close, visible only near the base of the plants. The trichomes measured 5.6–7.2 mic. in diameter. They tapered gradually from the base and ended in long hairs. The cell contents were homogeneous and blue-green.

Hollow Rock, Durham County, N. C. Free-floating and attached to dead twigs in a creek.

var. **maxima** var. nov. *Fig. 35.*

Except in size, the plants agree in every way with *Calothrix braunii*. With the plants under consideration, however, the diameter of the filaments may be greater by 4 mic., and that of the trichome by 2–3 mic. Also the sheath is occasionally yellowish, which is never the case in *C. braunii*.

Gaffney, Cherokee County, S. C. Forming a thin coating on the concrete bottom and sides of an outdoor lily pool located in the yard of the author's home.

GLOEOTRICHIA J. G. Agardh, 1842

Colonies macroscopic, at first attached, later often free-floating, solid when young, becoming inflated and hollow, soft, spherical or nearly so; filaments radiating from the center outward, repeatedly branched, false branches obvious only in very young colonies; sheaths conspicuous only near the base of the trichomes, being gelatinous and confluent in outer parts of the colony, sometimes yellow or brown, in some cases indurated with lime when mature; trichomes more or less torulose, tapering from base to apex; heterocysts usually basal, intercalary at points of branching; hormogones formed from upper parts of the trichomes, the apical hair being shed; hypnogonidia cylindrical, elongated, developed from the cell immediately above the heterocyst and remaining for some time within the basal part of the protecting sheath.

Gloeotrichia echinulata (J. E. Smith) Richter. Forschungsbr. Biol. Stat. zu Plön **2:** 31, fig. 1–8. 1894. (*Conferva echinulata* Smith. English Botany, pl. 1378. 1804.)

Free-floating filaments of these plants were collected in great abundance, they being no longer held together in colonies. The trichomes

measured 8–10 mic. in diameter at the base, tapering gradually from base to apex and ending in a long hair. Sheaths were distinct in the basal regions of the trichomes. The spherical heterocysts were terminal and measured 8–12.5 mic. in diameter. Granules were abundant within the cells.

Alligator Lake, Horry County, S. C. Not in distinct colonies, but forming slimy, mucilaginous masses attached to a species of *Chara*.

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EXPLANATION OF PLATES 14–17

(All figures × 750)

PLATE 14

Fig. 1. *Aphanothece microscopica* Naegeli. Only a few cells of the colony drawn in detail.
Fig. 2. *Microcystis aeruginosa* Kuetzing. Pseudovacuoles will be observed in these cells.
Fig. 3. *Gloeocapsa conglomerata* Kuetzing.
Fig. 4. *Aphanocapsa grevillei* (Hassall) Rabenhorst. Plants in different stages of division.
Fig. 5. *Phormidium crouani* Gomont.
Fig. 6. *Phormidium retzii* (C. A. Agardh) Gomont.
Fig. 7. *Chamaesiphon incrustans* Grunow.
Fig. 8. *Oscillatoria tenuis* Agardh. Apical portions of trichomes showing variations within the species. (Figs. after M. Forbes.)
Fig. 9. *Phormidium autumnale* (Agardh) Gomont.
Fig. 10. *Gomphosphaeria aponina* Kuetzing var. *delicatula* Virieux. Portion of a mature colony.
Fig. 11. *Aphanothece pallida* (Kuetzing) Rabenhorst. Several plants in various stages of division.
Fig. 12. *Chroococcus turgidus* (Kuetzing) Naegeli.

PLATE 15

Fig. 13. *Microcoleus vaginatus* (Vaucher) Gomont.
Fig. 14. *Stigonema turfaceum* (Berkeley) Cook.
Fig. 15. *Scytonema carolinianum* sp. nov.
Fig. 16. *Scytonema carolinianum* sp. nov. A portion of a filament showing agglutination of the false branches.
Fig. 17. *Scytonema crustaceum* Agardh.
Fig. 18. *Cylindrospermum muscicola* Kuetzing.

PLATE 16

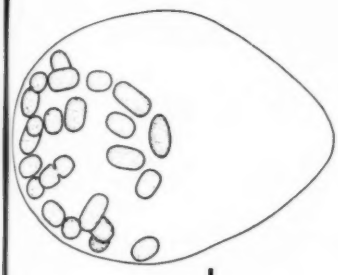
Fig. 19. *Schizothrix friesii* (Agardh) Gomont.
Fig. 20. *Cylindrospermum alatosporum* F. E. Fritsch. Two trichomes, one with a hypnogonidium in its early stage of development, the other with a nearly mature resting cell, and a series of five drawings showing successive stages in the germination of a hypnogonidium.
Fig. 21. *Nostoc ellipsosporum* (Desmaz.) Rabenhorst var. *minimum* var. nov.
Fig. 22. *Lynbya martensiana* Meneghini.
Fig. 23. *Nostoc punctiforme* (Kuetzing) Hariot. A single small colony.
Fig. 24. *Schizothrix aikenensis* (Wolle) comb. nov.
Fig. 25. *Anabaena parva* sp. nov. Several trichomes, each with developing hypnogonidia.

Fig. 26. *Cylindrospermum trichotospermum* Frémy.
Fig. 27. *Lyngbya aestuarii* (Mertens) Liebman.

PLATE 17

Fig. 28. *Tolypothrix delicatula* sp. nov. Portion of a single branching filament, and two heterocysts of different forms.
Fig. 29. *Hapalosiphon welwitschii* W. and G. S. West.
Fig. 30. *Calothrix genufleza* sp. nov. An entire filament, drawn in sections, epiphytic on a filament of *Lyngbya* sp.
Fig. 31. *Tolypothrix rechingeri* (Wille) Geitler.
Fig. 32. *Stigonema hormoides* (Kuetzing) Bornet and Flahault.
Fig. 33. *Anabaena torulosa* (Carm.) Lagerheim.
Fig. 34. *Anabaena laxa* (Rabenhorst) A. Braun.
Fig. 35. *Calothrix braunii* Bornet and Flahault var. *maxima* var. nov.

PLATE 14



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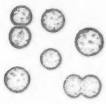

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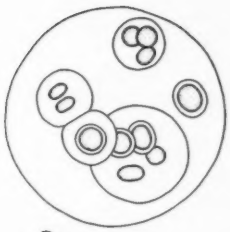

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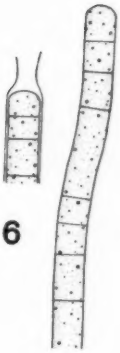

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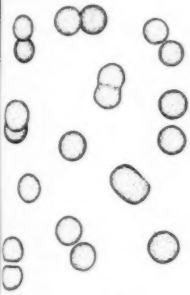

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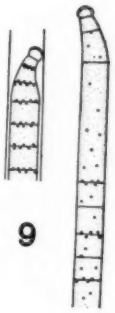

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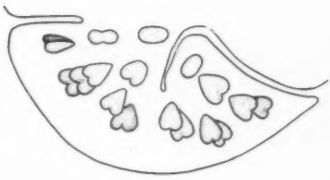

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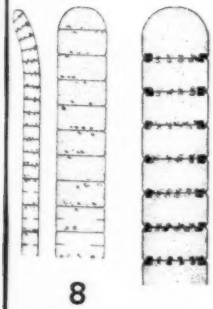

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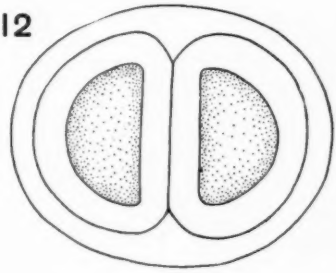

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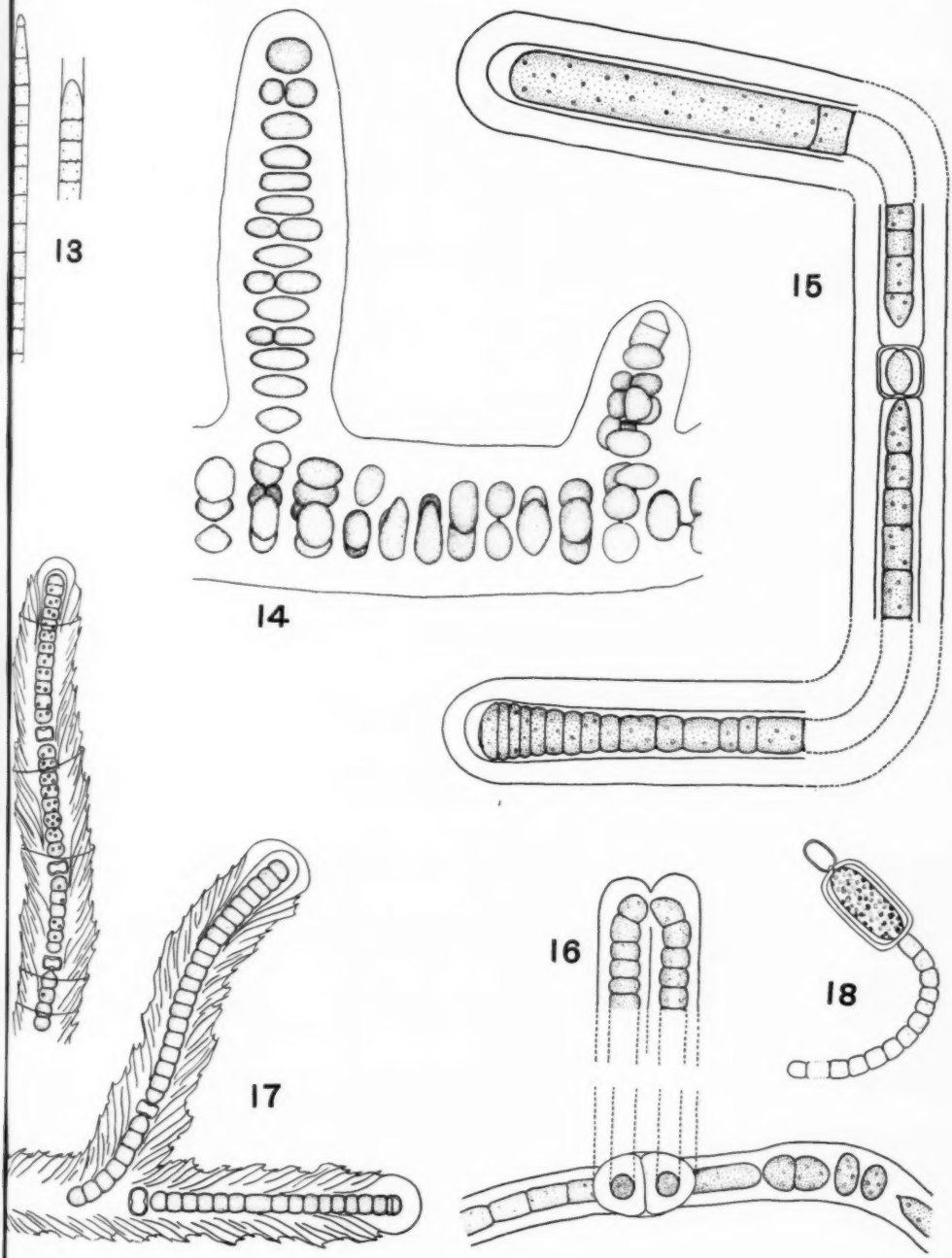
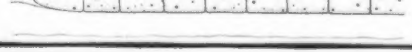
PLATE 15





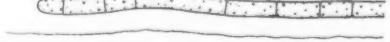
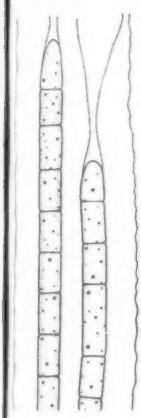
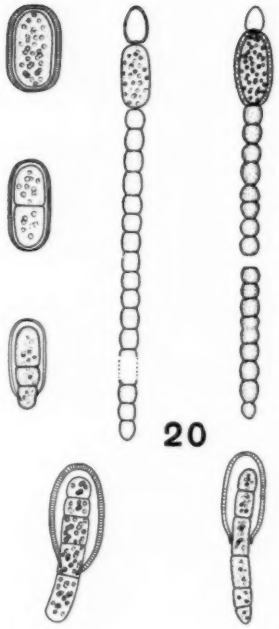
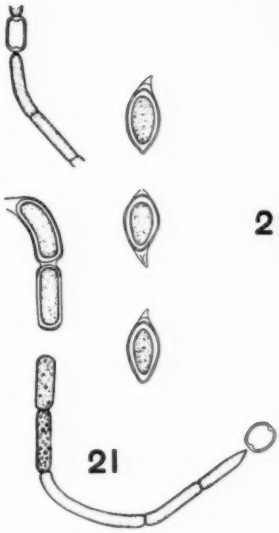
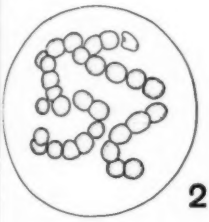


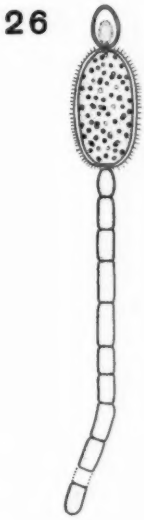
PLATE 16

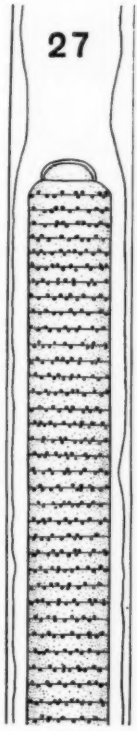












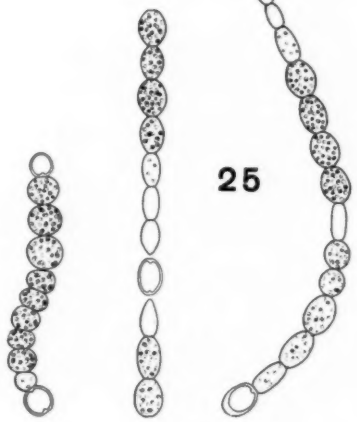


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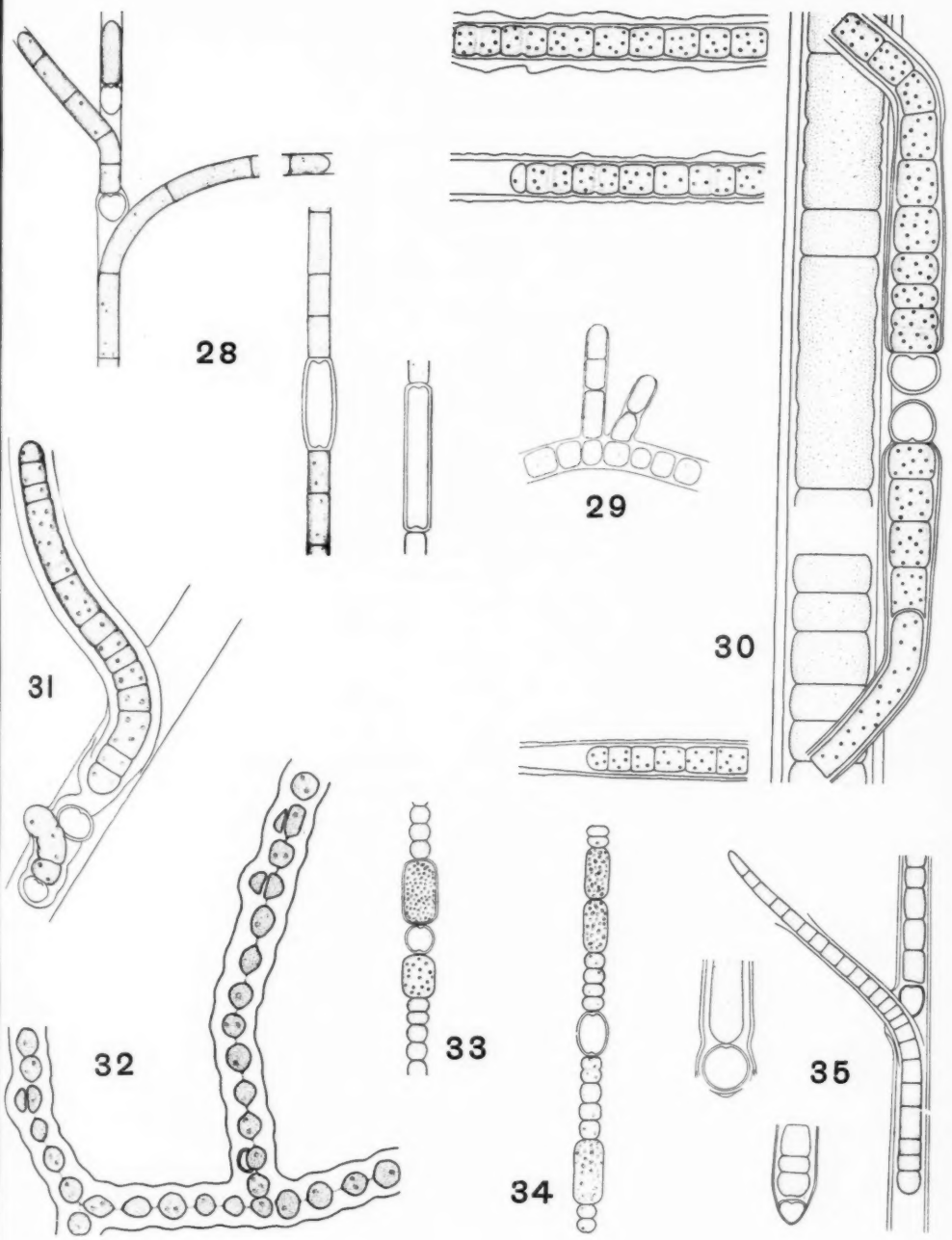
PLATE 17



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CORDYCEPS FROM THE MOUNTAINS OF NORTH CAROLINA AND TENNESSEE[1]

By E. B. Mains

Plates 18–21

The mountainous area of western North Carolina and eastern Tennessee appears to be very favorable for species of Cordyceps. In this paper, 20 species are reported. In 1910 Seaver (14) treated the genus for North America, recording 18 species. Thirteen species were reported for the United States. Several of these have since been shown to be synonymous and several new species have been described, the number of species now recorded for the United States being 21. In this paper 6 additional species are reported, of which 3 have not previously been described. Thus out of a total of 27 species for the United States 20 are here recorded for the mountains of North Carolina and Tennessee. *Cordyceps gracilis* Mont. & Dur., *C. clavulata* Schw., *C. acicularis* Rav., and *Ophiocordyceps macularis* Mains are not recorded but doubtless occur. *Cordyceps palustris* Berk. & Br., *C. insignis* Cooke & Rav., and *C. armeniaca* Berk. & Curt. are based on early collections and are known only from the types or have been rarely collected.

The writer's attention was directed to this area by the collections of Dr. A. H. Smith who spent parts of the summers of 1937 and 1938[2] with Professor L. R. Hesler studying the Agaricaceae of the Great Smoky Mountains National Park. He obtained many collections in other groups, among which were 50 belonging to 13 species of Cordyceps, one proving to be new.

Through the kindness of Dr. David H. Linder it has also been possible to study the collections of Professor Roland Thaxter now in the Farlow Herbarium of Harvard University. In 1887 and 1896, Professor Thaxter collected at Cranberry, North Carolina, and Burbank, Tennessee. He obtained many interesting collections of Cordyceps but

[1] Paper from the Department of Botany and the Herbarium of the University of Michigan.

[2] Dr. Smith's studies were supported by funds from the Horace H. Rackham School of Graduate Studies of the University of Michigan.

apparently did not publish the results of his studies. Thirteen species have come to the writer's attention, three having been recently described by Petch (13) and one being described as new in this paper.

Collections have also been received from Professor W. C. Coker of the University of North Carolina and Professor L. R. Hesler of the University of Tennessee which have given a number of additional records and one new species.

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2. Clavae present 4
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3. Perithecia developing from the apex of a buried pseudostalk *C. subsessilis* p. 122
4. Clavae very short, 1.5–2.5 mm., on spiders ..... *C. Thaxteri* p. 120
4. Clavae longer, on insects 5
5. Perithecia crowded, often adhering in groups 6
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6. Clavae ochraceous, asci cylindric, ascospores filiform, segmenting, on larvae of beetles ..... *C. variabilis* p. 123
7. Clavae yellowish white, soft, variable, on mature moths ..... *C. Sphingum* p. 121
7. Clavae orange, ochraceous, or brownish black 8
8. Clavae brownish black, club-shaped, on larvae of beetles ..... *C. Ravenelii* p. 122
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[3] This species has apparently not been collected in the area under consideration, but doubtless occurs.

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CORDYCEPS OPHIOGLOSSOIDES (Ehrh.) Link.

On *Elaphomyces* spp. Great Smoky Mountains National Park, Aug. and Sept. 1937 and 1938, A. H. Smith, common; Highlands, N. C., Sept. 1937, Aug. 1938, A. H. Smith (7503, 7521, 10390).

This is the most abundant and widespread species parasitizing *Elaphomyces*. The clavae are greenish brown to greenish black, club-shaped, the fertile portion cylindric or narrowly ellipsoid. The clavae are usually attached to the tubers by long yellow rhizomorphs. The perithecia are entirely embedded and are narrowly ovoid. The asci are cylindric, slightly narrowing at the apex and toward the base, 270–360 x 7–8 μ. The ascospores are filiform, nearly as long as the asci, multiseptate, early separating into the component cells which are short cylindric, 3–4 x 2 μ. There are specimens in the Farlow Herbarium collected by R. Thaxter at Burbank, Tennessee.

CORDYCEPS CAPITATA (Holm.) Link.

On *Elaphomyces* sp. Great Smoky Mountains National Park, Keener House, Aug. 3, 1938; Le Conte, Aug. 23, 1938, A. H. Smith (9666, 10475).

The clavae are olivaceous to greenish black, capitate, and are seated directly on the host. The heads are spherical and the perithecia are embedded. The asci are usually described as cylindric but in these specimens are narrowly fusoid, 320–390 μ long and 14–16 μ wide, narrowing at each end to 6–8 μ. The ascospores are multiseptate, filiform, nearly as long as the asci, 4 μ wide, tapering at each end to 2 μ. The ascospores early break into the component cells which are slightly fusoid, (16) 26–50 μ long, and 4 μ wide, narrowing to 2–2.5 μ at the ends. The end walls are somewhat thickened. This species varies somewhat in the size of the asci and specially of the part spores. In some collections the latter are much smaller, 14–30 x 2–3 μ, at one time being recognized as a separate species, *Cordyceps canadensis* Ellis and Everh.

CORDYCEPS INTERMEDIA Imai.

On *Elaphomyces* sp. Cades Cove, Great Smoky Mountains National Park, Aug. 18, 1938, A. H. Smith (10336).

This collection is very interesting. In its gross morphology and habit, it resembles *Cordyceps capitata*. The clavae are capitate and arise directly from the tubers. In the character of asci and ascospores it approaches *C. ophioglossoides*. The asci are nearly cylindric, 420–480 x 6 μ, narrowing only slightly toward the apex. The spores are filiform, nearly as long as the asci, multiseptate, the part spores short-cylindric, 3–5 x 1.5–2 μ. This species was described by Imai (3) from Japan and it is interesting that it should be found in the eastern United States. The clavae are smaller than those described from Japan but *C. capitata* shows a similar variation. It is possible that this species may be a hybrid between *C. capitata* and *C. ophioglossoides*.

CORDYCEPS THAXTERI sp. nov. Plate 18, figs. A–D

Clavis 2–10, pallide brunneis, 1.5–2.5 mm. altis, 150–180 μ crassis, sursum incrassatis 180–300 μ; peritheciis paucis, superficialibus, ovoideis, 960–1200 x 300–360 μ; ascis cylindraceis, 600–780 x 6–7 μ; ascosporis filiformibus, 1–1.5 μ crassis, multiseptatis, septis 6–10 μ distantibus; conidiosporis catenulatis, hyalinis, obovoideis, 4–11 x 2 μ.

In araneis, Cranberry, N. C., July–Aug. 1887, R. Thaxter (Farlow Herb. 4123, specimen typicum); Burbank, Tenn., Aug. 1896, R. Thaxter (Farlow Herb. 4122A).

This species is unusual in a number of respects. Collection 4123 contains approximately 70 small spiders covered with cottony masses of mycelium which attach the hosts to leaves. Each specimen bears

several pale buff clavae which are 1.5–2.5 mm. long, 150–180 μ thick below, usually enlarging above to 180–300 μ. The majority of the clavae bear only conidia which are produced in such quantity that they are often covered with large floccose masses. The conidia are catenulate and are developed on brown, slightly roughened, clavate cells which cover the clavae. The conidia are narrowly obovoid, 4–11 x 2 μ. It apparently is *Isaria arachnophila* Ditmar. Petch (8) has shown that the fungus most commonly reported as *Isaria arachnophila* is *Gibbelula arenarum* and that (9) the latter is the conidial stage of *Torrubiella Gibbelulae* Petch. Petch (8) has transfered *Isaria arachnophila* to *Hymenostilbe.*

Only a few, 1–4, perithecia develop on a clava. They are orange, conic-ovoid, and very large in proportion to the clavae, 960–1200 x 300–360 μ. The asci also are unusual for their size, 600–780 x 6–7 μ. The ascospores are nearly as long as the asci, 1–1.5 μ in width, multiseptate with the cells 6–10 μ long, tardily breaking into part spores.

The type is in the Farlow Herbarium of Harvard University, and in the Herbarium of the University of Michigan.

CORDYCEPS SPHINGUM (Tul.) Berk. & Curt.

On mature moths, Cranberry, N. C., Aug. 1887, R. Thaxter (Farlow Herb. 4054); Cranberry, N. C., Aug. 1896, R. Thaxter (Farlow Herb. 4053); Burbank, Tenn., R. Thaxter (Farlow Herb. 6192); Pretty Hollow, Haywood Co., N. C., Aug. 11, 1936, Lane Barksdale.

This is a very variable species which has been described under a number of names (10). In the above collections, the mature moths are covered with a matted mass of mycelium which also fastens them to the substratum. Some specimens are sterile or bear only conidia. Others lack clavae, and perithecia are produced on the matted mycelium covering the moth. When clavae are produced they vary considerably in number, form and size. They are soft, whitish, and often reach 10 mm. in length. The perithecia are scattered or crowded in groups. They are entirely superficial or immersed up to one-third of their height. They are brown, ovoid, 300–540 x 180–300 μ and contain cylindric asci 270–300 x 4 μ with filiform, multiseptate ascospores nearly as long as the asci, the part spores 4–6 x 1 μ.

There is some question concerning the first name which was applied to the perithecial stage. The Tulasnes (15) under the name *Torrubia sphingum* described perithecia from North American specimens. They cited *Isaria sphingum* Schw. for the conidial stage and *Akrophyton*

tuberculatum Lebert for the perithecial stage. Petch (10) concluded that Lebert's name should be used. Later he (10) has expressed doubt whether Lebert's name should apply to the North American fungus. It seems best for the present to employ the name used by both Schweinitz and the Tulasnes based on North American collections.

CORDYCEPS SUBSESSILIS Petch.

On larvae of beetles in rotten wood, Cranberry, North Carolina, 1887, R. Thaxter (Farlow Herb. 6155); Burbank, Tennessee, Aug. 1896, R. Thaxter (Farlow Herb. 6145).

Petch (13) described this species from the specimens cited above. The material is scanty and gives one the impression of poorly developed individuals. Petch states that it is doubtful whether the species should be described as a Cordyceps or a Torrubiella. He states that the species have a pseudostalk which may represent strands of mycelium bringing the fungus to the surface of the wood where perithecia are produced. They are described as free, crowded, narrowly flask-shaped, amber, 1–1.5 x 0.33–0.44 mm., containing cylindric asci 240 x 5 μ and part spores 3–7 x 1 μ.

CORDYCEPS RAVENELII Berk. & Curt.

On larvae of a beetle, Cranberry, N. C., Aug. 1887, R. Thaxter (Farlow Herb. 4050); Great Smoky Mountains National Park, Cades Cove, Aug. 18, 1938, A. H. Smith (10327).

These specimens have brownish-black, club-shaped clavae, 5.5 and 6.5 cm. long, 1.5 mm. thick, the fertile portion 2–2.5 mm. thick, the apices sterile, acute. The perithecia are superficial, free, closely grouped on the upper third, broadly ovoid, 400–480 x 300 μ. The asci are cylindric-clavate, 180–240 x 6–8 μ. The ascospores are cylindric, 160–190 x 2 μ, overlapping somewhat in the asci, multiseptate, the cells 22–30 μ long, tardily breaking into one-celled part spores.

This resembles *C. superficialis* in many characters. It differs in the more robust development, the darker color, and smaller more crowded perithecia. Petch (11) considers *C. Ravenelii* a synonym of *C. acicularis*. However, the latter has slender, ochraceous clavae which serve to distinguish it.

CORDYCEPS SUPERFICIALIS Peck.

On larvae of a beetle, Great Smoky Mountains National Park, Fighting Creek Gap, Aug. 22, 1938, A. H. Smith (10401); Highlands, N. C., Aug. 21, 1938, A. H. Smith (10395).

The clavae of these collections are grayish brown, up to 4.5 cm. long, 0.5–1.0 mm. thick. The perithecia are superficial, free, scattered and the apices of the clavae are sterile and acuminate. The perithecia are broadly ovoid to conic-ovoid, 480–600 x 420–480 μ, dark brown with grayish brown scales. The asci are cylindric, 200–270 x 6–8 μ. The ascospores are nearly as long as the asci, multiseptate, breaking into one-celled, cylindric segments, 16–32 x 1.5–2 μ.

This species was described by Peck (7) from New York. Seaver (14) placed it in synonymy with *Cordyceps acicularis*. The latter, however, has ochraceous clavae, clavate asci, and fusoid ascospores (5). It has been reported (5, 6) from New York, Michigan, Massachusetts, Connecticut, and Ontario.

CORDYCEPS MICHIGANENSIS Mains.

On larvae of beetles, Cullowhee, N. C., 1887, R. Thaxter (Farlow Herb. 6140); Great Smoky Mountains National Park, Keener House, Aug. 3 and 22, 1938, A. H. Smith (9667, 10397).

These specimens have several short slender clavae arising from the larvae. The clavae are ochraceous-orange, up to 3.5 cm. long, 1 mm. thick, the apices sterile and acuminate. The perithecia are superficial, free, scattered irregularly over the middle portion of the clavae. The asci are cylindric, up to 240 μ long and 6 μ wide. The ascospores are filiform, nearly as long as the asci, multiseptate, the cells 20–50 x 1.5 μ, somewhat tardily fragmenting. This species resembles *Cordyceps acicularis* in some respects, the latter differing in fewer, longer clavae, clavate asci, and fusoid ascospores. *Cordyceps michiganensis* has previously been reported (5, 6) from Michigan and Ontario.

CORDYCEPS VARIABILIS Petch. Plate 20, fig. D.

On larvae of beetles in rotten logs, Burbank, Tenn., Aug. 7, 1886, R. Thaxter (Farlow Herb. 6144); Great Smoky Mountains National Park, A. H. Smith; Indian Creek, Sept. 5, 1937 (7393), Aug. 14, 1938 10169), Sept. 2, 1938 (10834); Coon's Cove, Aug. 8, 1938 (9861); Alum Cove Trail, Sept. 1, 1938 (10772).

This species was recently described by Petch (13) from specimens from New York, Tennessee, Wisconsin, and Maine. As the name implies it is very variable. The specimens listed above have ochraceous clavae, 3–8 mm. long, 0.5 mm. thick. The perithecia are in small groups on one side of the clavae at various distances below the apices. They are superficial, adhering at their bases and sometimes for about

half their height. The asci are cylindric-fusoid, 200–330 x 6–8 μ, and the ascospores filiform, breaking into cylindric segments, 6–8 x 1.5–2 μ.

This species shows some resemblance to *Cordyceps viperina*. The latter has its perithecia embedded in a stroma.

CORDYCEPS VIPERINA Mains

On the larvae of beetles in rotten logs, Great Smoky Mountains National Park, Alum Cove Trail, Aug. 25, 1938, A. H. Smith (10543).

This species was recently described (6) from specimens collected in Nova Scotia, New York, Ontario, and British Honduras. This collection has ochraceous-orange clavae, 5–12 mm. long, 0.5 mm. thick. The perithecia are embedded in a pulvinate stroma which develops on one side of the clava some distance below the apex. The asci are cylindric-clavate, 200–250 x 8–10 μ, narrowing to 4–6 μ at the apex. The ascospores are filiform, multiseptate, early breaking into one-celled cylindric part spores, 6–8 x 2 μ.

CORDYCEPS UNILATERALIS (Tul.) Sacc. Plate 20, figs. A–C.

On ants, Cranberry, N. C., Aug. 1887, R. Thaxter (Farlow Herb. 4057); Cullowhee, N. C., June 15, 1896, R. Thaxter (Farlow Herb. 6161), June 1887 (Farlow Herb. 6190).

This interesting species produces clavae varying considerably in length, from a few mm. up to 20 mm. or more. They are very slender, black or brownish black, usually arising between the head and thorax. The perithecia are produced in pulvinate black stromata which may develop at various places along the clavae or occasionally may be terminal. Usually only a single stroma is produced although sometimes two or more may develop. The perithecia are fusoid-ellipsoid, 275–360 x 120–156 μ, entirely embedded in the stroma. The stroma between the upper portions of the perithecia is shrunken, giving the surface a verrucose appearance. The asci are clavate, 150–200 x 8–10 μ, and the ascospores, cylindric-fusoid, 132–162 x 2.5–3 μ, the ends acuminate. They are multiseptate, the septa 12–14 μ apart, not fragmenting.

CORDYCEPS MELOLANTHAE (Tul.) Sacc.

On large white grubs of beetles, Great Smoky Mountains National Park, A. H. Smith: Laurel Falls Trails, Aug. 8, 1938 (9893); Cades Cove, Aug. 16, 1938 (10245).

Each specimen has a single, pale ochraceous clava. They are irregularly clavate, somewhat furcate, 4.5–5 cm. long, 6 mm. wide below,

enlarging above to 12–15 mm. The perithecia are embedded in scattered irregular patches of stroma on the upper portion of the clavae. The asci are fusoid-cylindric, 180–240 x 6–7 μ, the ascospores filiform, nearly as long as the asci, multiseptate, soon breaking into cylindric segments, 4–6 x 1.5 μ.

This species has usually been reported as *Cordyceps herculea* (Schw.) Sacc. This name was based on *Sphaeria herculea* Schw. which Lloyd (4) has shown is a Gasteromycete *Cauloglossum transversarium* (see also 5, 12).

CORDYCEPS HESLERI sp. nov. Plate 19, figs. A & B.

Clava atra, 3 cm. longa, 5–7 mm. crassa; stipite griseo, 2 cm. longo; peritheciis fusoideo-ellipsoideis, 780–960 x 360–420 μ, immersis; ascis cylindriceis, 300–360 x 6–7 μ; ascosporis filiformibus; articulis ascosporarum, 3–4 x 1.5–2 μ.

In Cicada, Great Smoky Mountains National Park, Cades Cove, Blount Co., Tenn., Oct. 17, 1937, L. R. Hesler (Herb. Univ. Tenn, 10939).

The clava of this specimen is club-shaped, 3 cm. long (4.5 cm. when fresh). The stalk is 20 mm. long, 5 mm. thick, gray tinged with olive and yellowish at the base. The fertile portion is black and slightly thicker, 7 mm. The perithecia are entirely embedded, fusoid-ellipsoid, 780–960 x 360–420 μ. The upper layer of the stroma is made up of dark compact hyphae, the lower layer of hyaline, loosely woven hyphae. The upper layer in the dried specimen has shrunken and conforms to the outlines of the upper portions of the perithecia, giving the stroma a very rough appearance. The asci are cylindric, 300–360 x 6–7 μ, the ascospores filiform, nearly as long as the asci. The spores break into partspores 3–4 x 1.5–2 μ. The type is deposited in the Herbarium of the University of Michigan.

According to Petch (12) the various species described on Cicada are *Cordyceps sobolifera*. This species differs in a number of respects from *C. Hesleri*.

CORDYCEPS MILITARIS (L.) Link.

On pupae of lepidopterous insects, Great Smoky Mountains National Park, Aug. and Sept. 1937 and 1938, A. H. Smith, common; Highlands, N. C., Sept. 9, 1937, A. H. Smith (7506).

This is the most common species of Cordyceps. The orange, club-shaped clavae vary considerably in size, apparently correlated with the

size of the pupae from which they develop. The perithecia at maturity are only partially embedded in a soft stroma. The asci are cylindric, 400 μ or more long, 4 μ wide, the ascospores filiform, nearly as long as the asci, multiseptate, the part spores cylindric, 3–6 x 1 μ.

CORDYCEPS ELONGATA Petch.

On pupae and larvae of a moth *Apatela americana*, Cullowhee, N. C., R. Thaxter (Farlow Herb. 6277); Burbank, Tenn., Sept. 1887, R. Thaxter (Farlow Herb. 6134); Cranberry, N. C., 1887, R. Thaxter (Farlow Herb. 6135); Burbank, Tenn., Aug. 1896, R. Thaxter (Farlow Herb. 3906).

The clavae are 5–11 cm. long, slender, the stalks 0.5–1.5 mm. thick, light brown, the fertile portions cylindric, terete, 1.5–3.5 cm. long, slightly wider than the stalks, 1.5–2.5 mm. The stroma between the reddish brown perithecia is pale yellow. The clavae often terminate in a short, acuminate, sterile apex. The perithecia are embedded except for the upper third. They are ovoid, 420–480 x 240–276 μ. The asci are fusoid-cylindric, 240–300 x 7–8 μ, narrowing slightly toward the ends. The ascospores are filiform, nearly as long as the asci, 2–2.5 μ wide, slightly overlapping in the asci, multiseptate, not breaking into part spores. Three of the above collections were cited by Petch (13) in his description of the species. He also records a collection from Maine. Petch suggests that *Hirsutella gigantea* Petch is the conidial stage.

CORDYCEPS STYLOPHORA Berk. & Br.

On larvae of beetles in rotten logs, Burbank, Tenn., Aug. 1896, R. Thaxter (Farlow Herb. 4055); Great Smoky Mountains National Park, Alum Cave Trail, Aug. 22 and 26, 1938, A. H. Smith (10544, 11120).

These collections are immature. The clavae are grayish-brown, filiform to narrowly cylindric, up to 2.5 cm. long, the stipe 0.8 mm. thick, the cylindric stromata 1 mm. thick, the apices sterile, up to 5 mm. long, acuminate. This species is often collected in various degrees of maturity. Mature specimens have perithecia entirely embedded, the asci cylindric, 180–300 x 7–8 μ, the ascospores nearly as long as the asci, filiform, breaking into segments 6–10 x 2 μ. This species is fairly rare. It was first collected by Ravenal in South Carolina about 1857 (1). The next reported collection was by G. H. Hicks from Michigan in 1892 (2). Since 1934 it has been reported from Michigan and New York (5, 6).

CORDYCEPS DIPTERIGENA Berk. & Br. Plate 21, figs. A & B.

On flies, Cranberry, N. C., Aug. 1887, R. Thaxter (Farlow Herb. 4052, 5086, 6233c, 5077, Rel. Farl. 612); Burbank, Tenn., Aug. 1896, R. Thaxter (Farlow Herb. 6192).

The flies are covered with a yellowish mycelium by which they are attached to the substratum. One, two, and sometimes four clavae develop. These are 4–6 mm. long, capitate, the head hemispherical or turbinate, ochraceous-orange, punctate with brown ostioles, 1.5–2 mm. across. The stalks are slender, light buff. The perithecia are entirely embedded, directed upward with ostioles on the upper surface of the head, narrowly ovoid or fusoid-ovoid, 720–1000 x 240–540 μ. The asci are narrowly cylindric, 300–450 x 4–6 μ, the ascospores nearly as long as the asci, filiform, multiseptate, soon breaking into fusoid segments 8–12 x 1.5 μ.

This species has been rarely collected in the United States. There is a sterile specimen in the Mycological Collections of the United States Bureau of Plant Industry from Mississippi. Collections from Puerto Rico, Costa Rica, and Trinidad have been seen.

CORDYCEPS SPHECOCEPHALA (Klatzsch) Massee. Plate 21, figs. C & D.

On mature wasps, Great Smoky Mountains National Park, A. H. Smith: Elkmont, Sept. 7, 1937 (7478); Keener House, Aug. 3 and 8, 1938 (9664, 9938); LeConte, Aug. 17, 1938 (10305); Indian Creek, Sept. 5, 1937, and Aug. 14, 1938 (7399, 10176).

The clavae are capitate, 2–10 cm. long, the stalks light brown, slender, 0.5 mm. or less thick, the heads yellow, ovoid or oblong, 2–8 x 1.5–3 mm. The dried heads are irregularly longitudinally wrinkled. The perithecia are embedded except for the slightly projecting ostioles. The asci are cylindric, 240–300 x 5–6 μ, the ascospores filiform, nearly as long as the asci, multiseptate, breaking into part spores 8–12 x 1.5–2 μ.

This species has previously been reported from North America only from the West Indies. There is also a specimen in the Mycological Collection of the United States Bureau of Plant Industry from Florida.

CORDYCEPS SMITHII sp. nov. Plate 19, figs. C & D.

Clavis rubro-brunneis, 4–5 cm. longis, stipitibus 0.5 mm. crassis, capitibus conoideis vel ovoideis 4–5 x 1.5–2 mm.; peritheciis immersis, ovoideis, 240–260 x 150–160 μ; ascis clavatis, 90–120 x 8–10 μ; ascosporis fusoideis, 60–100 x 2.5–3 μ, multiseptatis.

In vespis, Great Smoky Mountains National Park, Keener House, Aug. 22, 1938, A. H. Smith (10396).

This specimen has two clavae arising from a wasp. They are reddish-brown, capitate, 4–5 cm. long, the stalks 0.5 mm. thick and the conic-ovoid heads 4–5 x 1.5–2 mm. The perithecia are embedded in the head and are ovoid, 240–260 x 150–160 μ. The asci are clavate, 90–120 x 8–10 μ, the ascospores fusoid, 60–100 x 2.5–3 μ, acuminate at each end and overlapping in the ascus. The spores are obscurely multiseptate and are not broken into part spores. The type specimen is deposited in the Herbarium of the University of Michigan.

Petch (12) in his discussion of the species of Cordyceps infecting bees and wasps described only one species with clavate asci and fusoid ascospores, *C. Humberti*. The latter, however, differs considerably in other respects from the above.

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EXPLANATION OF PLATES

PLATE 18

Fig. A. *Cordyceps Thaxteri*. Spider showing cottony mass of mycelium and clavae with perithecia. × 15.
Figs. B. & C. *Cordyceps Thaxteri*. Clavae bearing conidia. × 15.
Fig. C. *Cordyceps Thaxteri*. Perithecia. × 60.

PLATE 19

Fig. A. *Cordyceps Hesleri*. Cicada with clava. × 1.8.
Fig. B. *Cordyceps Hesleri*. Perithecia in stroma. × 60.
Fig. C. *Cordyceps Smithii*. Wasp with two clavae. × 1.5 (Photograph by A. H. Smith).
Fig. D. *Cordyceps Smithii*. Head with perithecia slightly projecting from the stroma. × 20.

PLATE 20

Fig. A. *Cordyceps unilateralis*. Ant with clavae, showing lateral stroma. × 4.
Fig. B. *Cordyceps unilateralis*. Head of ant with clavae showing terminal stroma. × 10.
Fig. C. *Cordyceps unilateralis*. Portion of head showing embedded perithecia. × 60.
Fig. D. *Cordyceps variabilis*. Two clavae with laterally grouped perithecia. × 10.

PLATE 21

Fig. A. *Cordyceps dipterigina*. Fly with four capitate clavae. × 5.
Fig. B. *Cordyceps dipterigina*. Portion of head of clava, showing perithecia. × 60.
Fig. C. *Cordyceps sphecocephala*. Wasp with capitate clava. × 2.
Fig. D. *Cordyceps sphecocephala*. Head showing ostioles of embedded perithecia. × 10.

PLATE 18

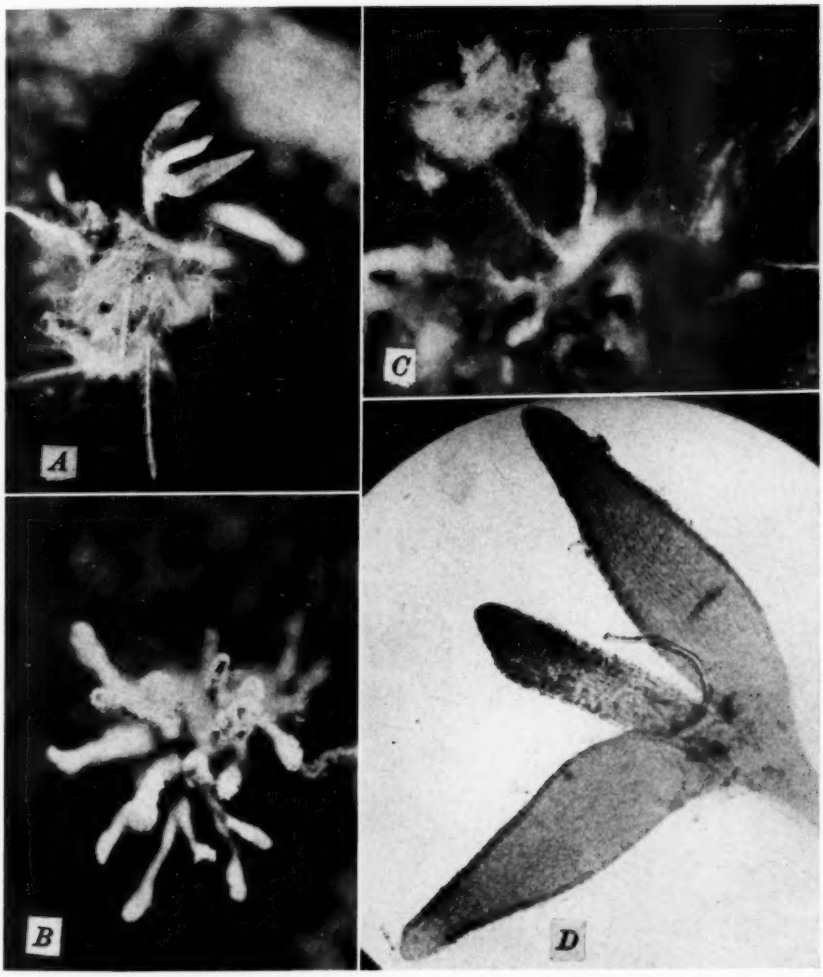

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PLATE 19

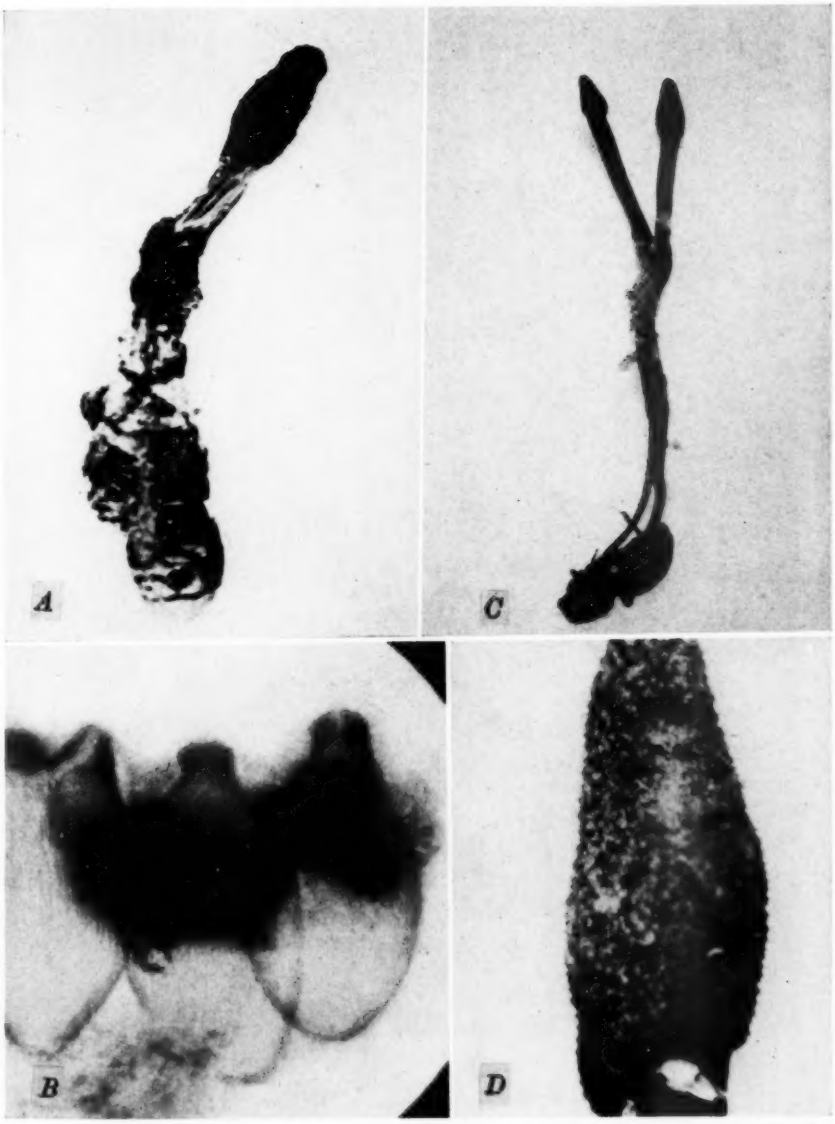

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PLATE 20

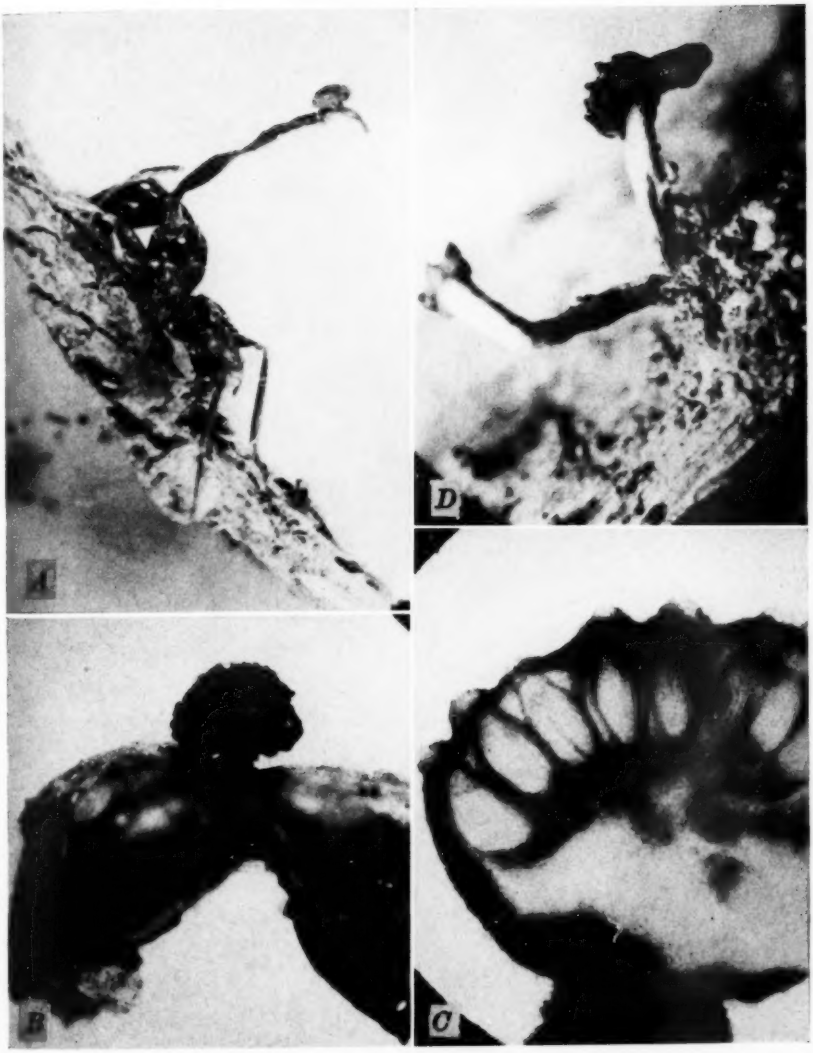
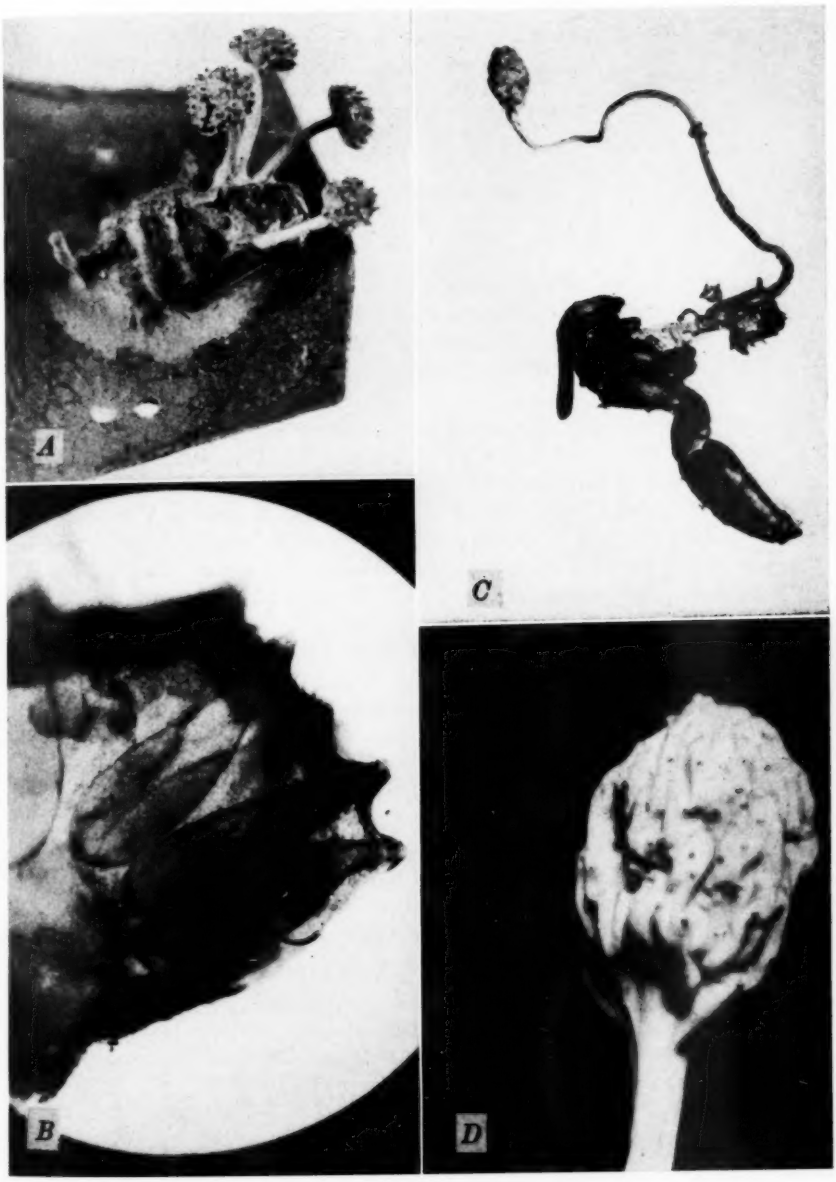


PLATE 21



A NEW SPECIES OF ELAPHOMYCES FROM THE GREAT SMOKY MOUNTAIN NATIONAL PARK[1]

By David H. Linder

One Text Figure

Through the kindness of Dr. E. B. Mains of the University of Michigan, the writer was enabled to study a species of *Elaphomyces* which was collected in the Great Smoky Mountain National Park by Dr. A. H. Smith. This species, represented by two fructifications, was parasitized by *Cordyceps intermedia* Imai.

The specimens of *Elaphomyces* are immediately characterized by their reddish-purple color, or "Prussian Red" of Ridgway,[2] and by their small dimensions. The specimens are further characterized by the gleba or "capillitium" which although white in a very small area at the very center, have outside of the central area a distinctly greenish-blue tinge which is made more pronounced when the specimen is wetted either with alcohol or water. Under the microscope, the fungus displays characteristics which place it in the subgenus *Malacoderma* Vitt. as recognized by Dodge[3] in his monographic study of the higher *Plectascales*. Within this subgenus the present specimens, according to descriptions, approach most closely to *Elaphomyces atropurpureus* Vitt. but a comparison with an authentic specimen of *E. atropurpureus* from Vittadini in the Patouillard Herbarium shows that there are striking differences in the color and important differences in the structure of the peridium, the peridium of the Vittadini specimen being somewhat sclerotic and about three times as thick as that of the Smoky Mountain material. Furthermore, the differences in the peridium become more marked when examined more closely under the microscope since the outer layer of the peridium of the latter specimens arises from the central layer, is composed of loosely interwoven hyphae (Fig. 1, a.) and only becomes densely organized in

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, No. 172.

[2] Ridgway, R. Color Standards and Color Nomenclature, Washington, D. C., 1912 was used wherever colors are cited within quotation marks.

[3] Dodge, C. W., Ann. Myc. **27** (3/4): 145–184, pl. 1–2, 1929.

the immediate vicinity of rootlets that are often included in the outer layer of the peridium. Unlike *E. atropurpureus*, the central layer is relatively narrow (Fig. 1, b.) and is only slightly sclerotized, while inside this median layer, and making up the bulk of the peridium is the inner peridium which is composed of large, loosely aggregated, rounded or somewhat angular cells (Fig. 1, c.) that are thin-walled and in mass "Light Pinkish Cinnamon." In contrast, then, to the thick hard layer to be found in *E. atropurpureus*, this species possesses a relatively slender, three-layered peridium of which the outer is of loosely interwoven purplish hyphae, a thin median layer of almost parallel hyphae in strands that are interwoven and are purplish, and finally an inner

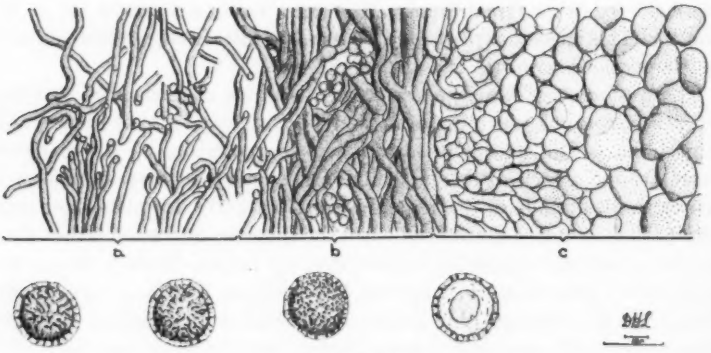


FIGURE 1

peridium of large thin-walled slightly brownish colored cells which give this layer, up to 500 μ thick, a punky texture.

Another species which approaches the present one is *E. nopporensis* Imai which was described from Japan and is the host upon which *Cordyceps intermedia* Imai was originally described. Although both the Smoky Mountain and the Japanese species of *Elaphomyces* are parasitized by the same species of *Cordyceps* and there is a resemblance between the two, they nevertheless are quite distinct. Grossly they differ in size, the American material being much smaller than the majority of the fructifications of the Japanese material, and the color more brilliant. Microscopically the two are quite distinct since the peridium of *E. nopporensis*, although relatively thin, is thicker than that of our material and, furthermore, the spores are larger, measuring (13.5)–15–16.5 μ in diameter, are considerably darker in color so that under the microscope

they appear a very deep chestnut color and the surface is densely and relatively even ornamented with close reticulations which frequently are broken into irregular, somewhat flattened, short, tooth-like echinulations. The spores of the Smoky Mountain material contrast with the others by their smaller dimensions, (6.6)–7.5–9 μ, their lighter color which is seldom darker than yellowish brown under the microscope, and, in spite of the fact that the spores are smaller than are those of *E. nopporensis*, the reticulations are approximately three or four times as coarse and the ridges or teeth, as the case may be, are nearly twice as long.

In view of the marked characteristics exhibited by this fungus, the writer considers it to be new to science and designates it as *Elaphomyces appalachiensis*.

Elaphomyces appalachiensis Linder sp. nov.

Fructificationes depresso-globosae, usque 6 x 5 mm. metientes; peridio externo "Prussian Red," hyphis 3–5 μ diametro, purpureis, laxe intertextis constante; peridio medio purpureo, hyphis subparallelis compactis plusminusve induratis et in funiculis intertextis, 120–140 μ crasso; peridio interno 300–500 μ crasso, "Light Cinnamon Buff," cellularum cum parietibus tenuibus composito, cellulis magnitudine variabilibus, bullatis vel pressu mutuo angulatis; gleba laxe gossypina, "Pearl Gray" siccata vel "Castor Gray" humecta; sporis sphaericis (6.5)–7.5–9 μ diametro, subhyalinis vel flavo-brunneis, subreticulatis vel reticulato-echinulatis.

Fructifications small, up to 6 x 5 mm., depressed globose, externally "Prussian Red." The peridium differentiated into three layers;—the outer peridium felty and composed of loosely interwoven hyphae which are 3–5 μ in diameter and purplish in color, derived from the middle peridium of purplish hyphae compacted in parallel strands which are closely interwoven to form a corneous prosenchymatous tissue 120–140 μ thick; and the inner peridium, "Light Pinkish Cinnamon" composed of thin-walled bullate or angular to loosely aggregated pseudoparenchymatous cells which are dilute brown. The gleba or "capillitium" is white in the center to "Pearl Gray" when dry or "Castor Gray" when wet. The ascospores are bluish in mass, appearing hyaline or dilute yellowish brown under the microscope, globose, (6.5)–7.5–9 μ in diameter, subreticulate to reticulate-echinulate.

Cades Cove, Great Smoky Mountain National Park, Tennessee, August 18, 1938, *A. H. Smith, 10,334*, parasitized by *Cordyceps intermedia* Imai. TYPE in the Farlow Herbarium of Harvard University and in the herbarium of the University of Michigan.

THE IDENTITY OF CERTAIN SPECIES OF THE SAPROLEGNIACEAE PARASITIC TO FISH[1, 2]

By Wesley N. Tiffney[3]

Although fungi parasitic to fish have been recognized since 1777 (cf. the excellent review by Ramsbottom, 29) and during the last 75 years there have been frequent reports from many parts of the world of such parasitic Saprolegniaceae, the exact identification of many of the species listed is doubtful. This is particularly true of such reports as that of Huxley (18) wherein the organism observed as a parasite of salmon was stated to be either *Saprolegnia torulosa* or *S. ferax*, or that of Clinton (6) who identified his fungus as either *S. mixta* or *S. ferax*. There have been many other cases of doubtful identification. In some reports not only were adequate descriptions and illustrations of the fungi lacking, but also the identifications, when made, were apparently without adequate basis, since in some instances, for example, the authors recorded the observation of zoösporangia and chlamydospores only and yet identified the fungi in question as species typically forming sexual organs on which their specific identity was based. More important yet, many authors have failed to recognize the need for single spore isolations to eliminate chance contaminants and saprophytes, as well as the need for inoculation experiments to verify the pathogenicity of the fungi they have reported as parasites. It is well known that a variety of Saprolegniaceous fungi may be isolated from almost any fresh water in nature, and the dead tissue around a fish's wound furnishes an admirable substratum for growth. Therefore in reporting a fungus as parasitic it is highly desirable to ascertain whether that fungus really does act as a parasite.

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University no. 158.

[2] A portion of a thesis presented to the Faculty of Harvard University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] The writer wishes to acknowledge his indebtedness to Professor William H. Weston, Jr., under whose direction this work was done, for his invaluable assistance, and to Doctor David H. Linder who gave many helpful suggestions during the course of the investigation. The writer is also indebted to many friends in the laboratory who aided him in collecting.

The taxonomy of these fungi is further confused by the fact that the range of variation of critical structures in some of the most common pathogens is not well understood, a situation which leads to the description of new species or varieties that upon more exact study are shown to be untenable.

The desirability of establishing with greater certainty the identity of the Saprolegniaceae parasitic to fish seems self-evident. The subject of parasitism is of considerable interest to biologists, and in addition these fungi are of considerable importance to fresh-water fisheries, for they are a constant possible source of difficulty in the artificial propagation of fish and under favorable conditions may even cause serious epidemics. Although the problem of parasitism in this group has received considerable attention, the difficulty of applying the work of others to the writer's findings is immediately apparent because of the uncertainty of identifications encountered. Therefore in connection with his investigations the writer found a study of the identity of the organisms involved to be of fundamental importance.

Since the object of this investigation was to study the taxonomy of the parasitic members of the Saprolegniaceae, it was desirable first, to collect from diseased animals as many strains of fungi as possible; second, to isolate single strains in pure cultures; third, to prove that these strains were capable of parasitizing animals; and finally, to study them taxonomically and compare them with the species described in the literature.

MATERIALS AND METHODS

Fungi were collected from the following sources: ponds and streams in and around greater Boston, South Weymouth, and Falmouth, Massachusetts, Guilford, Connecticut, and Rindge, New Hampshire, and aquaria in the laboratories of Harvard University, Cambridge, Massachusetts. Fungi were collected in the field during every month in the year when the water was not frozen, and several collections were made during the winter from fish in laboratory tanks supplied with running tap water. The hosts from which the fungi were collected belonged to three classes of vertebrates, Pisces (various fish), Amphibia (newts, young and adult frogs) and Reptilia (turtles).

The fungi were isolated only from diseased animals which were still living in order to avoid the inclusion of purely saprophytic strains. This precaution has not always been observed by students of parasitic fungi, an omission which has in some cases cast doubt on the validity of otherwise significant results. In the present study, considering the diverse

character of the hosts and the widely different environments in which they were found, as well as the three years over which collections were made, it seems justifiable to assume that the fungi collected comprise a fair representation of the parasitic members of the Saprolegniaceae in this region.

All strains were obtained in culture by transferring a tuft of fungus from the living animal to sterile water containing sterile hemp seed, for although parasitic the fungi grew readily as saprophytes on such substrata. The fungi in these gross cultures were then isolated from other filamentous fungi and freed from bacteria as much as possible by means of repeated washings and transfers in sterile water. The resultant subcultures, which were maintained on hemp seed in sterilized water, were apparently normal and vigorous and in no way different from those found in nature. In this way 128 isolations of pathogenic members of the Saprolegniaceae were made from diseased aquatic animals in southern New England.

A preliminary study showed that the 128 strains of parasitic fungi isolated belonged to three genera of the Saprolegniaceae, *Saprolegnia*, *Achlya*, and *Dictyuchus*. The genus *Saprolegnia* contained by far the greatest number for it included 124 of the strains collected; of these 122 lacked sexual organs and therefore might be assigned to *S. parasitica* Coker, while the other two had sexual organs whose characteristics placed them in the species *S. ferax* (Gruith.) Thuret. *Achlya* was represented by 3 strains, 2 of which were identified as *A. flagellata* Coker, while the third, which lacked sexual reproduction, was called for the time simply *Achlya* sp. The one member of *Dictyuchus* which was isolated also lacked sexual organs and was designated temporarily *Dictyuchus* sp.

The figures above show the remarkable frequency with which *Saprolegnia parasitica* was encountered. The data obtained in the course of these collections indicate that it is by far the most common and consequently the most serious Saprolegniaceous parasite of aquatic animals in this region, hence intensive study of this species seemed especially necessary. The 122 strains of *S. parasitica* were carefully studied and compared and it was found that 66 of them agreed with Coker's type species in every detail. The other 56 isolations were found to differ in various respects although they still might be considered to be within the concept of the species. Comparison of these 56 strains showed that they could be divided according to their morphological characteristics into 7 groups, each containing several strains. Since

the strains in any one group were apparently identical in every respect, an intensive study of them all seemed unnecessary, therefore one strain from each group was chosen for further study and material of each of the other strains was preserved for reference.

The strains to receive further study were immediately isolated in pure culture. This involved eliminating the ever-present bacteria and was accomplished by two methods, the first that of repeated washing and transfer as adapted from the methods of Klebs (23), Kauffman (21), Horn (16), and Pieters (27); the second that of ultra-violet irradiation of culture media as described by Blank and Tiffney (3). The second method was employed almost exclusively in the latter part of the work. After the fungi had been freed from bacteria, single spore isolations were made by means of the technique of repeated washing and spraying outlined by Weston (36). The strains derived from single spores were kept in stock cultures.

Since this work was an investigation of the parasitic members of the Saprolegniaceae, especial care was taken to verify the pathogenicity of the strains investigated by following the classic principles of Koch. The fungi had been obtained from living animals with the characteristic symptoms of fish mold disease and had been isolated in pure culture, thus fulfilling the first two postulates of Koch. Each strain of fungus was then used to inoculate healthy fish under controlled conditions and was found to parasitize the fish, producing the typical symptoms of fish mold infection and in many cases death.[4] Each strain was reisolated from the diseased fish and obtained in pure culture and its identity with the strain which had been used in inoculation was then verified, thus fulfilling the remaining postulates of Koch. By following this procedure the writer was able to eliminate any doubt about the pathogenicity of the strains which were the subjects of the taxonomic studies which follow.

For the purpose of comparative studies the fungi were grown at room temperature on hemp seed in sterilized pond water since it was found that this method of culture produced abundant and typical structures similar to those found in nature. Morphological studies were made on large amounts of representative living material derived from single spores. Care was taken to avoid the inclusion of atypical structures due to poor or congested conditions of growth, excessive age of cultures, and similar unfavorable factors. Comparative studies were made both of the qualitative morphologic features and of the sizes of vegetative

[4] A detailed description of the technique used in inoculating fish will be given in a future paper dealing with the host range of *Saprolegnia parasitica*.

hyphae, zoösporangia, zoöspores, chlamydospores, oögonia, oöspores, and antheridia, as well as the presence and relative abundance of these structures. Since genera in this family are distinguished on the non-sexual apparatus and species primarily on the sexual organs, all the data from these comparative studies were none too adequate for identifying these fungi by means of such authoritative monographs as Humphrey (17), Fischer (12), von Minden (25), Coker (7), Nagai (26) and Lund (24).

COMPARATIVE STUDIES

1. Saprolegnia

Saprolegnia is by far the most important genus of the filamentous fungi capable of parasitizing aquatic animals. In the literature 5 species of this genus, *S. ferax*, *S. torulosa*, *S. monoica*, *S. mixta* and *S. parasitica* (reported also as *Isoachlya parasitica*), and one variety, *Saprolegnia parasitica* var. *Kochhari*, have been reported as parasitic. *Saprolegnia ferax* and *S. parasitica*, both of which have been reported many times, are the important pathogens of the group, while the other three species are considerably less important since each has been reported only once. In fact, the inclusion of *S. torulosa* de Bary as a parasite may be questioned, for Huxley (18), who reported it, stated that the organism which he observed on salmon was either *S. torulosa* or *S. ferax*. The same is true of *S. mixta* de Bary for Clinton (6), author of the sole report, identified his parasitic fungus as either *S. mixta* or *S. ferax*. *Saprolegnia monoica* Pringsheim was reported once as a parasite, by Walentowicz (35), who described an epidemic among carp in Galicia, Poland, and stated that *S. monoica* was found on the fish in conjunction with *Achlya Nowickii*.

Saprolegnia ferax (Gruith.) Thuret is a recognized pathogen for it has been reported as parasitic by at least nine authors: Agersborg (1), Smith (32), Clinton (6), Hardy (13), Huxley (18), Stirling (33), Blanc (2), Schnetzler (31), and Collins (9). By the present writer two strains of this species were isolated, one from *Chelydra serpentina* and one from *Necturus maculosus*. Both were proved to be pathogenic by inoculation experiments and both formed sexual organs when grown on hemp seed in water cultures, bearing the typical oögonia and oöspores of the species. The identity of these two strains seems clear. Since *S. ferax* is a definite species which has been well described by Coker (7), Lund (24), and others it is not necessary to describe it here.

It is of interest to note, however, that the form obtained from *Necturus*

did not reveal its identity for a considerable period after its isolation. During this period it was tentatively assigned to *S. parasitica* because of a very close resemblance to that species, particularly in the number and appearance of the chlamydospores and the diameter of the cystospores (the encysted zoöspores). In *S. parasitica* Coker the cystospores are described as ranging from 9 μ to 13 μ with an average diameter of 10 μ, while in the form under consideration the cystospores ranged from 9 μ to 14 μ, with the greatest number having a diameter of 11 μ. However in regard to the type of zoösporangial renewal there was a marked discrepancy since in the writer's form renewal was accomplished usually by proliferation through the old sporangium and only occasionally by cymose branching, while in *S. parasitica* cymose branching was the predominant method. Although at first this difference might appear to be an important one the method of zoösporangial renewal will be shown to be of questionable diagnostic value in *S. parasitica* and hence not to be given too great significance here. When the form from *Necturus* was finally induced to fruit by growing it on a whole hemp seed with the seed coat punctured by only one small pin prick, a method used successfully by other authors, the characteristics of its sexual organs showed it to be *S. ferax*.

Saprolegnia parasitica Coker is probably the most common Saprolegniaceous parasite of aquatic animals. The species was established by Coker in 1923 to include the frequently encountered parasitic members of the genus, which, lacking sexual organs, could not correctly be identified with any of the species then described. The new species was distinguished chiefly by the facts that it was a parasite, that it did not form sexual organs but reproduced solely by asexual means, and that it often accomplished zoösporangial renewal by cymose branching as well as by proliferation through the old sporangium. Since its description the species has been collected by many authors. In the writer's isolations *S. parasitica* was the form most frequently secured, since 122 of the 128 strains isolated were identified as this species. From the remarkable predominance of this species it is reasonable to suppose that the sterile parasitic strains frequently mentioned in the literature and ascribed to various sexual species may have been the form now known as *S. parasitica*. Furthermore the occurrence of this species over an extremely wide geographical range, as indicated by the reports from North Carolina by Coker (7), from Bohemia by Cejp (4), from Japan by Nagai (26), from Denmark by Lund (24), and from India by Chaudhuri and Kochhar (5), as well as the remarkable frequency with which it

has been shown to occur in a restricted locality (the writer's collections in southern New England), indicates its prevalence and ubiquity. Its economic importance and the reason for the volume of literature on the subject are apparent if one adds that the fungus appears on a wide variety of hosts during every month of the year and under favorable conditions may cause destructive epidemics.

Saprolegnia parasitica was isolated by the writer from members of three vertebrate classes, Pisces, Amphibia, and Reptilia. The following species were found as hosts:[5] *Fundulus heteroclitus, Crassius auratus, Eupomotis gibbosus, Perca flavescens, Ameiurus* sp., *Erimyzon* sp., *Necturus maculosus, Triturus viridescens, Rana pipiens,* and *Chrysemys concinna.* The fungi were collected throughout the year under a variety of natural and artificial conditions. As has been stated previously the 122 strains collected were cultured, purified and divided into 8 groups, each containing a number of apparently identical strains; one strain from each group was chosen as its representative. (The first group was represented by a subculture of the type culture of *S. parasitica* Coker which was deposited by Coker in the Centraalbureau voor Schimmelcultures, Baarn, Holland.) These 8 representative strains showed distinct differences even upon superficial examination although they all possessed the characteristics of sterility and pathogenicity typical of *S. parasitica.* The differences they exhibited, however, emphasized the desirability of comparative studies to determine the range of variation possible within the species.

Since sexual organs were lacking in these strains it was necessary to resort to studies of their asexual reproductive structures and vegetative growth. Comparisons were made of the qualitative morphological features and sizes of the vegetative hyphae, zoösporangia, zoöspores, and chlamydospores. In general the strains showed no essential variations in qualitative characteristics and it was necessary to make measurements of various structures in order to demonstrate their differences. The results of these measurements, which are given in Table I, are in every case based on at least 100 observations and are presented in the form "11–22–38" in which the first and last numbers represent the extremes observed and the middle number represents the measurement obtained most frequently. These figures represent random selection from a large quantity of living material grown and studied under similar conditions, as described previously. The total diameter of growth was

[5] Teleostei determined from Jordan and Evermann (19); Amphibia and Reptilia from Pratt (28), and Ditmars (11).

determined by measuring the mature seven-day colonies grown on hemp seed in water. To determine comparative sizes of zoöspores the cystospore stages were measured because they were stationary and spherical, whereas the primary and secondary swarming stages were motile and comparatively irregular in shape. Such features as the method of zoösporangial renewal and the shape and arrangement of chlamydospores were determined by the examination of large amounts of representative material.

The significant results of these studies on the 7 representative strains of *S. parasitica* isolated by the writer and on a subculture of Coker's type culture of the species, for comparison, are presented in Table I. The writer wishes to emphasize the fact that these measurements are not in any sense intended as an exact quantitative analysis of the features involved, but merely as a convenient and definite way of comparing their sizes.

The vegetative hyphae of all strains were essentially alike in qualitative characteristics, but they did exhibit some differences in size as the measurements of the first three characters listed in Table I will show. The total diameter of colony growth on hemp seed (which is of course an expression of hyphal length) varied slightly in the different strains but the range of variation was so small that it seemed to have no significance for the purposes of this study. Considerably greater variation was found in the diameter of the hyphae at the base and at the tip, the dimensions being twice as large in some strains as in others. Another feature which is not listed in the table but which was evident upon observation of the fungi was the tapering shape characteristic of the hyphae of some strains. Strain 3, in which the hyphae were most frequently 40 μ in diameter at the base and 9 μ in diameter at the tip, had the most conspicuously tapering hyphae, while Strain 1 (Coker's type culture) and Strain 11, in both of which the diameter of the base of a hypha was in general only 4 μ greater than the diameter of its tip, had hyphae that did not taper, and the other 5 strains formed a transitional series between these two extremes.

The differences in vegetative growth discussed above have no apparent diagnostic significance, however, for a survey shows that although the fungi can be arranged in a series on the basis of any one of the above characters, the arrangement is different for each character and there is no evident correlation between them. It would seem therefore that the slight differences exhibited in the vegetative growth of these strains have no diagnostic value.

TABLE I

Comparative Morphological Studies of Strains of Saprolegnia parasitica

CHARACTER	STRAIN 1, SUBCULTURE OF THE TYPE	STRAIN 3	STRAIN 4	STRAIN 6	STRAIN 8	STRAIN 11	STRAIN 12	STRAIN 16
7 day colony on hemp seed, diameter in mm.	9–12–16	9–12–16	5–13–18	9–13.5–21	9–10–13	7–9–11	10–12–15	9–12–16
Vegetative hyphae, diameter in μ								
At base	12–21–29	23–40–55	15–23–33	25–44–54	18–36–59	18–25–36	25–44–54	14–28–48
At tip	11–17–30	6–9–12	10–16–23	15–26–46	15–24–30	11–21–34	15–26–46	7–10–15
Zoösporangia								
Length in μ	135–260–469	70–108–160	118–205–262	250–465–775	155–231–440	95–185–338	247–407–893	98–160–220
Width in μ	13–21–38	6–11–14	9–12–26	11–24–49	20–34–45	19–25–30	26–36–53	11–18–27
Renewal by proliferation thru old sporangia	0–20%	70%–100%	0–20%	0–20%	0–30%	0–20%	40%–60%	85%–100%
Renewal by cymose branching	80%–100%	0–30%	80%–100%	80%–100%	70%–100%	80%–100%	40%–60%	0–15%
Cystospores, diameter in μ	9–10–13	8–10–11	9–10–12	9–11–12	9–10–12	8–10–13	9–11–14	6–8–10
Chlamydospores								
Abundance	Abundant	Abundant	Sparse	Abundant	Abundant	Abundant	Moderately abundant	Sparse
Position	Terminal, intercalary, or in cymosely branching chains	Terminal, intercalary, or in cymosely branching chains	Terminal, or intercalary	Terminal, intercalary, or in cymosely branching chains	Terminal, intercalary, or in cymosely branching chains	Terminal, intercalary, or in cymosely branching chains	Terminal, intercalary, or in cymosely branching chains	Terminal, intercalary, or in chains
Shape	Globose to clavate	Globose to clavate	Globose, rarely clavate	Globose to clavate	Globose to clavate	Globose to clavate	Globose to clavate	Globose to clavate

One would expect to find more definite and constant characteristics upon studying the zoösporangia, especially since the methods of formation and discharge of these structures are of considerable importance in the classification of the Saprolegniaceae. In the species under consideration Chaudhuri and Kochhar (5) have made a difference in zoösporangial length the distinguishing characteristic of a new variety, *S. parasitica* var. *Kochhari*, while Nagai (26) has even transferred *S. parasitica* to another genus, *Isoachlya*, because of one of its methods of zoösporangial renewal. A comparative study of the characteristics of the zoösporangia of Coker's type culture of the species and the 7 strains collected by the writer would therefore seem to be of considerable interest.

No significant differences in the shapes of the zoösporangia of the 8 strains were noted. They all exhibited some irregularity in shape at times, a feature of this species which has been observed by Coker (7), Lund (24), and others, but there was nothing which would serve to distinguish one strain from another.

However, the 8 strains did display marked differences in the sizes of zoösporangia, and the results of measurements of their lengths and widths are presented in the table. The zoösporangia of the 8 strains ranged in length from a minimum of 70 μ in Strain 3 to a maximum of 893 μ in Strain 12. This is a maximum range greater than any that has yet been set for this species. However, if the 8 strains are arranged according to their sporangial lengths as shown below it will be evident that the measurements overlap markedly and form a continuous series with no conspicuous gaps.

S. PARASITICA—STRAIN NUMBER	LENGTHS OF ZOÖSPORANGIA IN μ		
	Minimum	Most frequent	Maximum
3	70	108	160
16	98	160	220
11	95	185	338
4	118	205	262
8	155	231	440
1 (Coker's type)	135	260	469
12	247	407	893
6	250	465	775

If an investigator were to collect a culture like Strain 3 and another like Strain 12 without seeing the intervening types it would be quite

possible for him to assume that he had two different varieties of the same species. On the other hand, a series such as the one above in which the measurements overlap as they do presents an entirely different picture. The writer believes himself justified in assuming that he is dealing with one species whose range of variation upon intensive study has appeared to be much greater than the original descriptions would lead him to believe. It is his contention that this range of variation is not a sufficient basis for the establishment of new varieties, but rather should be considered as an extension of the present range of variation within the species.

In 1935 Chaudhuri and Kochhar (5) in a survey of the water molds of India established a new variety, *S. parasitica* var. *Kochhari* Chaudhuri, on the basis of zoösporangial length. These authors described the length of *S. parasitica* Coker as ranging between 280 μ and 560 μ, while the length of the new variety of Chaudhuri was stated to be 400 μ to 600 μ, a range much more restricted than any the present writer has found in the forms he has studied. According to the evidence presented above the range of zoösporangial length in *S. parasitica* should be extended to include forms ranging from 70 μ to 893 μ. Consequently it is the writer's opinion that the variety *S. parasitica* var. *Kochhari* Chaudhuri has been established on an inadequate basis.

Zoösporangial width was found to vary in proportion to length, for the width was in general from 5% to 15% of the length. Therefore the arrangement of strains as presented on zoösporangial length would also hold to an equal degree for zoösporangial width.

The method of zoösporangial renewal, whether by growth through the old sporangium or by cymose branching from below the base of the old sporangium, is often considered an important feature in the classification of Saprolegniaceous fungi. One of the distinguishing characteristics of the species *S. parasitica* is that it shows cymose branching, "very often proliferating from the side below as in *Achlya*" (Coker, 7, p. 58). Kauffman (22) gave even greater weight to this character, for in 1921 he made it the chief basis for the formation of the genus *Isoachlya*, saying, "The genus is characterized and distinguished, in the main, by the presence of the cymose or *Achlya* mode of formation of secondary sporangia, coupled with diplanetic zoöspores" (Kauffman, 22, p. 231). Coker recognized the genus *Isoachlya* but nevertheless did not place *Saprolegnia parasitica* in it when he described this species two years later. Subsequently Nagai (26) and Lund (24) both pointed out that according to the definitions of the genus and the species, *S. parasitica*

Coker should be placed in *Isoachlya* and Nagai made this change, calling the organism *I. parasitica* (Coker) Nagai. Coker (8), however, in the *North American Flora* still retains the species in the genus *Saprolegnia*.

In the course of the writer's studies, however, this character proved to be of less diagnostic value than might be expected, as an examination of the data presented in Table I will show. In Coker's type culture (Strain 1) and Strains 4, 6, and 11 zoösporangial renewal was generally accomplished (in 80% to approximately 100% of the cases) by cymose branching and rarely (in 20% to almost none of the cases) by growth through the empty sporangium. In Strain 8 cymose branching was somewhat less frequent, including approximately 70% of the cases. In Strain 12 renewal was effected about equally by the two methods. In Strain 3 only about 30% of the renewal was accomplished by cymose branching, while growth through the old sporangium was the predominant method. In Strain 16 cymose branching was rare and renewal was accomplished almost wholly (85% to almost 100%) by proliferation through the old sporangium. It will be seen that the 8 strains formed a transitional series from one extreme, in which renewal was effected almost completely by cymose branching, to the other, in which it was accomplished almost completely by growth through the old sporangium, with intergrading forms between. As has been stated before, the writer found no valid reason for excluding any of the strains studied from the species *S. parasitica;* consequently he believes that the data presented above indicate that the limits of this species should be extended to include those forms in which cymose branching is not necessarily the predominant mode of zoösporangial renewal.

In view of the facts presented above the writer sees no necessity for transferring *S. parasitica* to the genus *Isoachlya*. As these studies show, the *Achlya*-like cymose branching is not a conspicuous feature of all strains of the species. Moreover, cymose branching as one method of zoösporangial renewal is an accepted feature in several species of *Saprolegnia*, i.e., *S. ferax* (Gruith.) Thuret, *S. asterophora* de Bary, *S. spiralis* Cornu, *S. diclina* Humphrey, *S. anisospora* de Bary and others (Coker 7). To the writer, therefore, it seems preferable to retain *S. parasitica* in its original genus.

Furthermore, it seems fitting to inquire at this point whether the method of renewal of zoösporangia, which may vary to a marked degree within the confines of one species, is a valid character upon which to base generic distinctions. An exact study of several members of the genera involved is highly desirable and should do much to clarify the situation,

for it seems probable that greater significance has been attributed to the mode of zoösporangial renewal in certain forms than the present knowledge of this character and the amount of exact study devoted to it would warrant.

In the method of discharge of zoöspores no significant differences between the 8 strains were noted. Occasional irregularities occurred, particularly when conditions had become somewhat unfavorable, but this is a recognized occurrence and has no importance for the purposes of this study. Upon discharge the spores swarmed about, at length coming to rest as spherical cystospores, from which the secondary spores later escaped and swam about in the manner typical of the genus *Saprolegnia*. In a few instances spores were observed to go through repeated swarming periods. This phenomenon has been observed by other writers (Weston, 36, Höhnk, 15).

Comparative sizes of the zoöspores were determined by measuring the cystospores, since they were stationary and spherical and therefore could be measured with more accuracy than the active swimming spores. Little variation was shown in cystospore size, as may be seen from Table I. The cystospores of 7 of the strains closely approximated one another, ranging from 8 μ to 14 μ in diameter, being most frequently 10 to 11 μ. Only Strain 16 showed some difference, having cystospores that ranged from 6 μ to 10 μ in diameter, being most frequently 8 μ.

Chlamydospores or resting bodies, while one of the most noticeable features of these fungi in culture, were disappointing as sources of diagnostic characters. Some slight differences between the various strains were observed in the abundance and position of these bodies. They were sparse in Strains 4 and 16, moderately abundant in Strain 12 and abundant in the 5 other strains. In addition, Strains 4 and 16 lacked the cymosely branching chains of chlamydospores that were present occasionally in Strain 12 and frequently in the other strains. These differences, while interesting, are hardly marked enough to justify much significance being given them, especially since they show no correlation with the various characteristics previously described.

None of the strains formed sexual organs under any conditions, consequently comparative studies were limited to the asexual features discussed above.

It is interesting to note that Kanouse (20) has reported a case which seems to be parallel with the findings of the writer in some respects. Her organism, isolated from fish eggs and identified as *S. parasitica*, although typically asexual did respond to certain salt solutions and

produced fruiting bodies. One of the writer's forms, which was isolated from *Necturus* and appeared to be *Saprolegnia parasitica* failed to fruit in the same salt solutions described by Kanouse, but did fruit finally when grown on hemp seed, and was identified as *S. ferax*. Both forms in their asexual stages agreed with Coker's description of *S. parasitica* and both upon fruiting exhibited the characteristics of two entirely different species. It seems to the writer that these cases are indicative of the possibility of a curious situation in *S. parasitica*, namely, that this "species," defined on asexual characters, may in reality be composed of forms of heterogeneous origin which, if they could be induced to fruit, might be found to belong to several different species within the genus *Saprolegnia*.

Since the individual significance of each of the writer's findings has been discussed in the foregoing pages there is no need for repetition here. Taken as a whole these studies lead to the conclusion that while *Saprolegnia parasitica* shows considerable variation in its characteristics there is nevertheless no valid ground for dividing it into more than one species, and therefore the writer believes that for the present these findings should be interpreted only as extending the range of variation possible within the species. The rather unsatisfactory nature of this conclusion illustrates clearly the difficulty of defining a species on asexual characters alone.

The results of these studies lead one to speculate on the nature of the species *S. parasitica*. It may be that, like certain members of *Dictyuchus* studied by Couch (10), these sterile strains of *Saprolegnia* are in reality segregated unisexual strains which when combined with complementary strains should result in sexual reproduction. On the other hand, it is possible that these fungi, which do not reproduce sexually under the usual cultural conditions, may be induced to fruit when the proper combination of environmental factors is found. Finally, if neither of these suppositions can be demonstrated we shall be forced for the present to conclude that the organism is a species which under ordinary conditions has lost the power of sexual reproduction. For the present it seems best to consider *Saprolegnia parasitica* a species of convenience, and it should be treated as such until further studies have demonstrated its exact nature.

2. Achlya

The genus *Achlya* contains four species which have been reported as parasitic, the identity of which is unquestioned since in every case the

fungus formed sexual organs which permitted accurate identification. *Achlya prolifera* (Nees) de Bary appears most often in the literature, for it has been reported by Robin (30), Schnetzler (31), and Blanc (2). *Achlya racemosa* Hildebrand was observed as a parasite by Humphrey (17) and by Hine (14) who also reported *A. polyandra* Hildebrand. It might be noted that none of these three species was observed on more than a few individual hosts, and none was demonstrated to be pathogenic by inoculation experiments.

Achlya flagellata Coker was found as a parasite by Tiffney and Wolf (34) in Massachusetts and Wisconsin, respectively. Wolf observed a serious epidemic among trout in a Wisconsin fish hatchery; *A. flagellata* was found to be the cause of the epidemic and no other fungi were involved. The present writer reported two isolations of this species from diseased animals, one from *Triturus viridescens* on which the fungus occurred in conjunction with *Saprolegnia parasitica*, and one from *Lebistes reticulatus*. The second strain was used to inoculate *Fundulus heteroclitus* and was shown to act as a wound parasite.

In addition, the writer succeeded in isolating a sterile strain of *Achlya* from the outer portion of the shell of *Chelydra serpentina* where it was associated with *Saprolegnia ferax*. Inoculation experiments showed this strain, like the *A. flagellata* mentioned above, to be capable of a parasitic existence only if the host's resistance had been lowered by severe injury, a fact which places it in the category of wound parasites. This sterile *Achlya* was maintained in culture for approximately a year and a half, and during that time was subjected to a variety of environmental conditions but could not be induced to fruit. Since this organism, being non-sexual, cannot with certainty be referred to a recognized species it seems desirable to include a brief description of it here.

Achlya sp.

Growth comparatively slow, requiring 7 days or more to reach its greatest extent; mature colony 5–15 mm. in diameter on hemp seed; hyphae 30–70 μ in diameter at the base, tapering to 20–27 μ at the tip; zoösporangia numerous, 200–420 μ in length and renewed by repeated cymose branching; cystospores 10–12 μ in diameter; chlamydospores spherical to clavate, single or in chains, occasionally reaching a diameter of 100 μ or more.

This genus has not, heretofore, been considered particularly important as a parasite of aquatic animals, but it seems probable, especially in view of the serious epidemic reported by Wolf (Tiffney and Wolf, 34), that future observations may disclose the genus to be more important in this respect than has been supposed.

3. *Dictyuchus*

The genus *Dictyuchus*, as far as the writer is aware, contains no species which have been reported as parasitic to animals.

One form belonging to this genus was isolated by the writer from a living *Triturus viridescens*. Inoculation experiments showed the fungus to be a parasite, but like the species of *Achlya* previously described it could be classed only as a wound parasite. This organism never formed sexual fruiting bodies although it was subjected to a variety of cultural conditions over a period of ten months. In view of the findings of Couch (10) it seems probable that this form is a member of the species *Dictyuchus monosporus* Leitgeb.

From the foregoing report it is apparent that the writer found *Saprolegnia* to be far the most important genus of the fungi parasitic to fish, since it was so frequently isolated. *Achlya* was found to be considerably less important and *Dictyuchus* of no real importance in this respect. This survey, by reason of the time devoted to it and the area covered, probably gives a reliable index to the situation in New England. It would be most interesting to have similar surveys from other regions for purposes of comparison.

SUMMARY

128 strains of Saprolegniaceae were isolated in southern New England from living diseased aquatic animals.

The representative strains of fungi which received intensive study were isolated in pure culture as single spore strains and proved to be pathogenic by inoculation experiments.

2 strains of *Saprolegnia ferax* were obtained. One of them, which in its asexual stage closely resembled *S. parasitica*, was induced to fruit only by special cultural methods.

122 strains of *Saprolegnia parasitica* were collected. Comparative studies of a subculture of Coker's type culture of this species and representative strains isolated by the writer showed that the more or less marked variations displayed in vegetative and asexual reproductive structures should not be considered to be of diagnostic value, but should serve only to extend the range of variation possible within the species. In the writer's opinion *S. parasitica* should be considered a species of convenience until further study has demonstrated its exact nature.

The variety *S. parasitica* var. *Kochhari* is considered to have been established on an inadequate basis, since the characteristic upon which

it was based is included in the range of the species as it is interpreted here.

The character "renewal of zoösporangia by cymose branching" was shown to be more variable and correspondingly less significant than has been thought; consequently the writer prefers not to accept the transfer of *Saprolegnia parasitica* to the genus *Isoachlya*, but to retain it in its original genus.

2 strains of *Achlya flagellata*, 1 *Achlya* sp., and 1 *Dictyuchus* sp. were isolated and shown to be wound parasites. This is, to the writer's knowledge, the first observation of a member of *Dictyuchus* parasitic to animals.

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A REMARKABLE SAPROPHYTIC FUNGOID ALGA*

By W. C. Coker and Leland Shanor

Plates 22 and 23

In the spring of 1937 the senior author, while scouting in upper Chatham County, about 7½ miles from Chapel Hill, North Carolina, noticed a white fluffy fungoid-looking growth in a small spring stream near the base of a large sawdust pile which had been burning for at least 4 months. This growth was attached to objects in the stream such as leaves, roots, sticks, and rocks, but more frequently to blades and panicles of grass which were bent over into the running water (Figs. 19, 20). Thinking that it was probably *Leptomitus*, one of the well known water molds that we have been observing for years, material attached to grass and twigs was brought into the laboratory for study. On examination this proved to be not a water mold but a remarkable plant that is the subject of this paper.

These first collections made were kept in the laboratory for a short time, but soon died from the increased bacterial contamination. Again in the fall of the same year, a visit to this location showed that the plant was still available, and on this same excursion a second station was found about two miles from the first, our plant growing again under similar circumstances. In the spring of 1938, the plant having been found growing abundantly in this latter stream, it was decided to study it more carefully. This work was started on April 23, 1938, and cultural studies begun on April 30.

If this plant had been green, we would have said at once that it was probably a *Stigeoclonium* or a close relative of that genus in the Chaetophoraceae. The size and whole organization of the plant body is remarkably similar to that of members of this group. Each individual plant body consists of a main axis, generally tapering upward and branching at rather regular intervals from the upper ends of the cells, these rebranching again several times and finally ending in long slender

* A preliminary report on this paper was presented before the National Academy of Sciences, meeting at Chapel Hill, North Carolina, October 25, 1938.

cells which taper to extremely fine points (figs. 1, 2, 4). The basal cell or cells give off numerous irregular and branching rhizoidal holdfasts (fig. 7, 8, 24), just as in *Stigeoclonium* and its relatives. The attachment of these plants seems to be entirely for support and not for nutrition, as is indicated by experiments cited below.

The cells of this plant appear perfectly transparent in transmitted light and there is no trace of a body that might be called a colorless chromatophore, if one could use such a contradictory term. The cytoplasmic lining of the large cells is confined to the cell walls and is extremely thin, in fact hardly observable with ordinary magnification, being usually less than 1.5μ thick except in the ends of the cells. In the young more narrow cells the protoplasm is not confined to the region of the cell walls but extends here and there across the interior giving a very vacuolated appearance. The nuclei in the largest cells are large and obvious, measuring from 5.6–8.6μ in diameter, and have a very conspicuous gleaming nucleolus (figs. 7, 25). Nuclei in small tip cells measure 1.7–2μ in diameter. In a cell of average size, when seen in surface view after being killed in the fumes of osmic acid and stained with gentian violet, the cytoplasm seems to be arranged in inconspicuously diagonal strands reminding one of the arrangement of the chloroplasts of *Nitella* (fig. 6). In large old plants a number of the basal cells and the rhizoidal holdfasts arising from them appear to be dead and empty. We have been unable to demonstrate a nucleus in the holdfast cells.

GROWTH IN NATURAL ENVIRONMENT AND CHEMICAL ANALYSIS OF THE BRANCH WATER

At our first station, the spring supplying the branch is located only about 20 yards above the upper end of the burning sawdust pile. The fluffy growth of our plant appeared at the uppermost point that seepage from the pile would likely enter, and above this line, which was a sharp one, it was entirely absent. From this point downward it was abundant for many yards. Perhaps 30 or 40 yards below this seepage, the branch disappeared in a thick tangle of *Smilax* and was not examined further.

At the second station, the spring was located in a very similar position in relation to the burning sawdust pile as it was at the first, but after about 50 yards, this small stream from the spring entered a larger branch and the growth of our plant in this stream is quite remarkable. Growth

in the smaller stream began just opposite the sawdust pile and continued down this and on into the larger one. Above the point in the large stream where the small stream entered there was no sign of any growth. Likewise, no trace of growth could be noted in the smaller stream above the seepage line. The larger stream here is filled with large rocks and these were abundantly covered with this fluffy growth, sometimes to a depth of as much as 5 cm. (fig. 19), in addition to the usual abundance of it found attached to roots, twigs, grass blades and panicles. Growth attached to grass has been observed in this stream as far as about ½ mile from the point where the smaller stream entered it.

Cool temperatures favor the growth of this plant, and we have been unable to find it in the warmer seasons. Most of the collections have been made either during the late fall and winter or during the spring months. Our last collection made during the summer of 1938 was on June 26. On September 15, no trace of growth could be found at either station and on other visits on September 27 and October 6, still no growth could be seen. On October 13, a few blades of grass with a small amount of growth attached were collected at our second station, but not until October 24 was there an abundance of material available.

The fact that no growth appeared above the seepage point in both of these stations indicates clearly that some nourishing substance finds its way into the water from these burning sawdust piles which is essential to the growth of the plant. Further evidence that the nourishment for growth is obtained from the water and not from the substratum is shown by the fact that growth on rocks in the stream below the seepage is equal to that of plants attached to organic objects. The attachment of these plants to objects in water is, then, evidently for support alone. Still further evidence that the nourishment comes from the burning sawdust is the following observation. Sometime during the past summer the sawdust pile at the first station quit burning, and when the growth reappeared late in October at the second station, none could be observed in the stream where it was first found. Although there has been an abundant growth in the stream at the second station all during the winter and spring months, the plant has not again reappeared at the first station.

Members of our chemistry department very generously undertook to analyse the branch water in which the plants were growing. We are greatly indebted to Dr. Edward Mack, Jr., Dr. R. W. Bost, and Dr. E. C. Markham for their most valuable assistance in this work. The

results of their analysis, so far as it was thought necessary to carry it, is as follows:

pH 598, not much more acid than distilled water.

	mg. per liter
Total solids (dried at 100 C.)	126
Total solids (after burning)	47
Chlorine (Cl)	5
Iron	1
Nitrogen (as free NH)	0.04
Nitrogen (albuminoid)	0.6
Nitrogen (total organic)	0.5

Results when one liter was evaporated to 25 cc. and tested for various possible organic substances:

1. $FeCl_3$ test was negative, showing no tannins or phenols.
2. Indophenol test negative, showing no phenols.
3. Bromine water, test negative, showing no unsaturates of phenols.
4. Schiff's test was positive, showing no aldehydes (sugars do not respond to this test).
5. Molisch test was positive, showing carbohydrates.
6. Quantitative determination of reducing sugars calculated as glucose, 6.9 mg. per liter.
7. Permanganate titration of reducing substances calculated as glucose, 6.0 mg. per liter.

This analysis showed that the branch water was almost free from mineral content but contained a noticeable amount of reducing sugar. It also has a very characteristic acid sweet odor.

REPRODUCTION AND DEVELOPMENT

To our surprise, as well as to theirs, the chemists found that after filtering the water brought in with the plants through a hard filter which would usually remove all visible particles, in a few days a fluffy growth was seen throughout the water which gave it a milky appearance. We have found that the ultimate delicate tips of the end branches of this plant are very easily thrown off, so that if handled at all these separated tips would always be found floating freely in the water. These tips may be single-celled or be made up of as many as seven or eight cells (figs. 2, 3). It was inferred that some of these tips must have passed the filter.

Additional samples were then filtered in our laboratory, and 100 cc. samples of this filtrate centrifuged for three minutes to separate out any

tips that might have passed through the filter. Samples drawn from the bottom of the centrifuge tube after this treatment showed, when examined with the compound microscope, that a few of these tips had been drawn through the filter. The tips in the filtrate were mostly single-celled but a few were as much as three cells long. They could not be detected in the filtrate with the unaided eye.

Experiments begun by us to study this growth more closely showed unexpectedly that the very tip points of a great number of these cells were glutinous and would instantly stick to any solid object touched. This was first noted when very young blades of grass were placed in cultures to determine if our plant would live on these. It was noted almost immediately that these small tip branches would stick to the grass, and even though these were moved around in water, the attached tips would sway about but would not become detached.

In a dish in which many of these tips are floating about, a string or blade of grass after being drawn through the water will, upon examination under the compound microscope, show a number of these tips sticking to it. This glutinous property possessed by some of these cell-tips was further demonstrated when water containing many of them was placed under the high magnification of the binocular microscope. A needle touched to the tip of these branches then drawn through the water revealed that the tip-branches would be drawn along without becoming detached. Considerable shaking about in the water was often necessary to dislodge them. Not all of these tip-branches possessed this glutinous quality but most of the several-celled ones did, as did also a number of the single-celled ones. These short single-celled branch tips were the type that most frequently passed through the filter.

When left in a petri dish undisturbed for a few hours, a number of these tips can be seen attached to the bottom of the dish by the point end and standing upside down. Agitating the water only causes them to sway around the point of attachment but does not loosen them. After a few days, when a few granules of sugar were added, these plants were observed to have formed a holdfast system from this basal pointed cell, the upper part had grown considerably and had become branched, and these small plants were now rapidly assuming the appearance of more mature ones. By growing in this way, the polarization of growth is reversed.

Under normal conditions in nature, it seems obvious that these branch-tips, after becoming free in the water, are carried by the current

until the pointed tip cell comes in contact with some object in the stream. They then stick and proceed with their development. On a firm surface the basal cell soon forms a holdfast apparatus by flattening out and branching (figs. 9, 10, 11, 13, and 14). Later, other branches are given off by a few cells immediately above, which likewise grow downward, become attached to the surface and thus form a very substantial holdfast system (fig. 7 and 8). In mature plants this holdfast system reminds one of the prop roots developed by Indian corn. On soft tissue such as young tender grass shoots, these young plants may send their first simple holdfasts into the tissue of the substratum (fig. 12), but that this growth is not for nourishment is shown by the fact that the plant will not grow unless sugar is added. When sugar is present, growth is as active on rocks and glass as on organic material.

CULTURE EXPERIMENTS

Since the chemical analysis of the branch water in which the plants were growing showed the presence of a considerable amount of glucose sugar, our first effort to culture this plant was in a sugar solution. The blades of grass to which the material was attached were divided into three approximately equal parts and placed in fingerbowls in distilled water. One of these was left on the desk at room temperature, one placed in the icebox but no sugar added, and the third placed in the icebox and a few grains of sugar (dextrose) added each day. The culture to which sugar was added began to grow immediately and after three weeks had reached its maximum growth. Before any sugar was added the growth of the plant extended out about one centimeter from the blade of grass to which it was attached and at the end of this period when measured again, had increased to about $2\frac{1}{4}$ cm. Soon after this the culture had to be disposed of because of excessive bacterial contamination.

After about a week, the culture which had been placed on the desk had become completely destroyed by bacterial activity. In the icebox the bacterial action was retarded by a lower temperature so that, although the culture to which no sugar had been added showed very little if any growth, it was not completely destroyed after the three-week period.

Two weeks later more fresh material was collected and additional cultures made using several concentrations of dextrose in the water. The amount of sugar used was 3.5, 7, 10, 14, and 20 mg. per liter of distilled water. In all of these concentrations except the 3.5 mg. per

liter solution the growth was discernible after a week's time, but bacteria could not be eliminated from cultures made in this way so they had to be discarded. No further culture attempts were made until after October 15, 1938, when repeated efforts finally resulted in obtaining growth on agar that was entirely free of bacteria.

After we had obtained good growth of bacteria-free cultures on agar, we undertook again to try culturing this plant in a solution. Because growth was relatively good on maltose-peptone #5 agar, maltose-peptone solutions were made up without the agar. Solutions of 0.2 and 0.3 per cent dextrose were also prepared and these were placed in two liter Erlenmeyer flasks and autoclaved. To each flask was added a small block of an agar culture containing fresh growth on December 1, 1938.

After 24 hours, these showed that the plant had grown out quite noticeably from the block of agar that had been used for the inoculation, and when held up to the light, showed numerous tip cells floating about in the solution. Very few of these tips were seen at this time in the 0.2 and 0.3 per cent dextrose solutions as compared to the number in the maltose-peptone #5 medium.

After three days, the maltose-peptone solution began to appear slightly milky, and when held up to the light, the solution was found to be full of these minute tips floating about. A few had already settled to the bottom of the flask and were beginning to branch, as were many of those floating about in the solution. The flasks containing the dextrose solutions never became so full of these floating tips nor was such an abundant growth produced later as took place in the maltose-peptone solution. These tip branches continued to grow and, as they increased in size, settled to the bottom of the flasks.

This growth continued unobserved for several weeks during the Christmas recess, but after a month's time, the bottoms of the flasks were found covered to a depth of a little over an inch with the white fluffy growth of this plant (fig. 21). Most of the dividing branch-tips had also settled to the bottom so that the upper part of the solution no longer had a milky appearance. After this very little change was noted in the amount of growth, and by March 1, 1939, the only difference seen was a collection on the bottom of the flasks of a very slightly brownish mass somewhat more dense than the upper more fluffy portion. When this was examined it was found to be only a dense aggregation of short fragmenting branch-tip cells.

The appearance of the plants grown in the maltose-peptone #5

solution is quite similar to that of plants found in nature. The branching is very much the same and the general structure appears to be identical. However, some slight variation has been noted in the shape of the cells. The cells on the main axis of these plants show considerable enlargment at the nodes as had been noted before in cultures that had become badly contaminated with bacteria (figs. 5, 25). The cells of the side branches also frequently show considerable enlargement at the nodes, particularly on the end of the cells at which branching takes place. This type of enlargement gives these cells somewhat of a drumstick appearance (fig. 15). The cells of the side branches continually produce one to several-celled branches which are liberated into the solution.

The behavior of these reproductive tip-branches is different in solution from those of material brought in fresh from the stream. None of these tips seem to possess sticky points and could not be induced to stick to a needle drawn through the water, as could those brought in from the field. They also do not become attached to the glass but either continue to grow in the solution or fragment to form a number of shorter cells which grow into multicellular branched plants or just increase in size and fragment again. This fragmentation takes place after the cell has divided by the rounding up of adjoining ends of the two new cells (fig. 18). This rounding of the ends of the cells continues as the cells enlarge so that the two new cells either separate entirely or remain attached to each other with the ends continuing to elongate. Because of this tendency to stick together, several cells are frequently found which remain attached to each other but which are growing and dividing as if growing separately. Often before branching takes place in one of these branch tips, the cell at one end becomes very long and thread-like (fig. 17), suggesting perhaps an attempt at holdfast formation. Sometimes the tip cells on much branched plants in solution will become elongated and thread-like in a similar fashion before being set free by the parent plant (fig. 16).

GROWTH ON AGAR

In our early attempts to obtain growth of this plant on agar, the material was so completely polluted with bacteria that all efforts to obtain it in pure culture failed. In a later attempt, some plants began to grow along with a yellow bacillus-type bacterium which seemed to interfere in no way with the growth of the plant. Eventually pure cultures free of bacteria were obtained by cutting off tips of branches which had grown

away from bacteria under the surface of the agar. When we first attempted to grow cultures on a solidified medium, 3% agar was used for this purpose. When this failed to give good results, the possibility of the medium being too solid was suggested. When the amount of agar was reduced to 1.2%, growth was more satisfactory. Potato-dextrose, Blakeslee's # 230, corn meal, 0.2% dextrose, and our maltose-peptone # 5 agars were all prepared with this concentration of the agar. The potato-dextrose and Blakeslee's # 230 seemed too strong and no growth took place on either of these. Fair growth resulted on the 0.2% dextrose but the best results were obtained on the maltose-peptone # 5 agar.

As soon as reproductive tips are placed on agar, the cells divide and begin to grow. After 24 hours, many have become several-celled and have even begun to branch in some cases. The tip cell elongates, becomes narrow and much bent without forming any crosswalls, and grows down into the agar. This tip cell is the one which would have given rise to the holdfast system had this branch become attached to some object in water. Each small plant thus started continues to grow on the agar, the cells enlarge and branches arise from the anterior end of each cell near the joint with the adjoining cell as do the plants found in nature in the running water. These branches very seldom grow out and become more branched as do the hyphae of cultures of fungi, but rather grow more parallel to the main axis, become separated from the parent cell, and are pushed aside by another branch from the same cell. Many cells are, therefore, produced along the main axis which lie parallel with it. Each cell or group of cells that formed a branch in this way continues to grow and produce branches as did the first axis. In this way whole bands of branching strands are formed in which the larger axis is in the middle and the smaller branching side axes are found around this (fig. 23). The large cells of the central axis are very conspicuous in a band of growing threads.

Growth on agar may be either on the surface or under the surface (fig. 22). Growth on the surface produces a raised, white, glistening colony with the greater amount of growth at the point of inoculation with raised bands extending outward. Under the agar the bands are not quite so compact. A surface band always is accompanied by some growth down into the agar. Whether the growth is on the surface or down in the agar, the main axis does not extend straight but seems to meander somewhat in a zig-zag fashion. This is perhaps due to the plant's growth against the resistance of the agar and the elongation of

each cell in place after it was first cut off. This winding growth is very evident in surface growth and is shown clearly in fig. 23. If one of these surface strands is removed and placed in a drop of water, the plants straighten out and appear almost exactly as plants removed from their attachments in the field. They have no holdfasts, of course, but otherwise there is no detectable difference.

Frequently when branches from a colony grow downward through the agar, they attempt to form holdfasts against the bottom of the petri dish (fig. 26). These holdfast-like branches, however, are seldom divided into cells and do not approach the more complex holdfast apparatus of plants grown attached to objects in water. Holdfast-like structures have not been observed formed in the agar except where a growing point has touched the bottom of the petri dish.

The growth of this plant on agar is very slow. Pure culture transfers made by cutting out small blocks from an original agar culture or by transferring a strand of the surface growth, may produce a colony in a month which rarely has a radius of more than an inch from the point of inoculation. The white glistening accumulated growth in the center of these colonies is very suggestive of the appearance of bacteria or yeast colonies.

COMPOSITION OF THE CELL WALL

The cell wall was first tested for cellulose by the usual chloro-iodide of zinc treatment. A few plants were placed on a slide in a drop of water and with them a few hyphae of *Achlya flagellata* and cotton fibers, the walls of which show perfectly the cellulose reaction with this solution. When the water was drawn off, and the reagent added, the walls of our plant became only faintly yellowish while the hyphae of the *Achlya* and the cotton fibers turned a beautiful violet-blue color. As a further test, the procedure outlined by Chamberlain (Methods in Plant Histology, 5th revised ed., p. 85) for demonstrating the presence of cellulose was followed. Here again the walls of our plant yielded only a yellowish color while the walls of the *Achlya* and the cotton fibers became a deep blue. These two tests indicated to us that cellulose was either not present in the walls of this plant or that these tests for some reason were prevented from indicating it.

We then tried Zander's test for chitin as given in Lee (Microtomist's Vade-Mecum, 10th ed., p. 600) but this failed to give a positive reaction. When treated with gentian violet, the walls became stained with this dye.

Samples of our plant brought in from the stream and carefully cleaned, and samples taken from the surface of agar cultures were dried and very generously analysed for us by Dr. J. C. Andrews of the Department of Biological Chemistry. We are indebted to Dr. Andrews for the following results: Hydrolysis gives glucose, and the glucosamine test was negative. When tested for nitrogen 2.80% was obtained. This would correspond to a maximum of about 36% chitin if *all* N were in that form. This would, likewise, correspond to a maximum of about 17.5% protein.

This material gave a positive biuret test and positive tests for tyrosine and tryptophane. A test for cystine showed only a very slight amount of this substance.

The above results indicate that the walls probably do not contain chitin but rather a carbohydrate related to cellulose, the composition of which is at present unknown.

TAXONOMY

In regard to its relationships to other algae, an extended discussion seems unnecessary. Except in this case, there is no saprophytic plant known that can be convincingly compared to any Green Alga group. It is true that Printz in Engler and Prantl (Pflanzenfamilien, 2nd ed. **3**: 252. 1927) suggests the Monoblepharidaceae as colorless relatives of the Oedogoniaceae, but if there is any such relationship, it is more than obscure. A comparison of our plant with parasitic and endophytic algae suggests no parallels. It may be of significance that *Stigeoclonium tenue*, a green plant, has been found attached to living fish in a fishpond at Melbourne, Australia, and in a small pool at Tanabe, Japan (Tilden, Algae and their Life Relations, p. 458).

The peculiar nature of this plant makes it extremely difficult to fit into any scheme of classification. From its vegetative structure it would seem best to consider it a highly peculiar derivative of the algae, with its nearest relatives in the higher forms of the Chaetophoraceae. The differences would seem sufficient to place it in a family of its own, which might best be placed near the Chaetophoraceae as an aberrant member of the order Ulotrichales.

SAPROCHAETACEAE n. fam.

Colorless saprophytic plants with branching and general form of the Chaetophoraceae; attachment to substratum by means of a complicated rhizoidal holdfast system; reproduction, as far as known, solely by a vegetative detachment of the branch tip cells.

One genus, *Saprochaete*.

SAPROCHAETE n. gen.

With the characters of the family. One species.

Saprochaete saccharophila n. sp.

Plants occurring attached to objects in running water, appearing milk white in mass, reaching a total length usually of about 2 cm., under some circumstances up to 5 cm. Plant body complicately branched from an attached base, branches arising from the upper ends of the cells, ultimate branchlets tapering to a sharp point; axis cells up to 175μ long by 33μ in diameter, cylindrical, sometimes having enlargements at the cell junctions, nuclei in largest cells conspicuous, up to 8.6μ in diameter and having a prominent nucleolus; chlorophyll and plastids entirely lacking; cell wall not responding to tests for cellulose or chitin; propagation entirely by vegetative means, the distal cell or cells of the branchlets becoming free, then attaching themselves by their glutinous tips and growing backward, reversing polarity.

Found only in running water in which a considerable amount of reducing sugar is present. Collected many times during cooler months from the spring of 1937 to 1939 at two stations in Chatham County, N. C., growing attached to objects in spring runs receiving seepage from burning sawdust piles.

SUMMARY

A remarkable plant having the form of a higher green alga and the nutrition of a saprophytic fungus is reported from two stations in northern Chatham County, N. C., growing in small spring runs receiving seepage from burning sawdust piles. It has a body closely resembling that of *Stigeoclonium*, but is entirely without chlorophyll or any trace of an organ resembling a chloroplast. The cytoplasm is confined to an extremely thin layer under the wall in the larger cells, and each of these cells has a single large conspicuous nucleus. The tip cells of the branches are extremely tenuous and end in a fine point which has been found in most cases to be viscid. These tip cells fall off easily and become attached by their points to objects in the water. Reversing their polarity, they soon develop into much branched plants reaching a length of 1 cm. or more and having the appearance to the naked eye of a vigorous water mold. This method of vegetative multiplication is the only type of propagation known for this plant so far. A complicated group of rhizoidal holdfasts is sent off from the basal cell and the cells above it. The cell walls do not give the usual positive reactions for either cellulose or chitin with standard tests, but a chemical analysis indicates a closer affinity to cellulose.

Pure culture isolations have been grown on agar containing small amounts of sugar, and in weak sugar solutions. The appearance of the growth in culture and any deviations from the typical structure of plants found in nature are discussed. For nutrition, this plant seems to be dependent on the presence of reducing sugars, as is indicated by its growth in nature in water in which reducing sugars are present and from cultural studies in sugar solutions in the laboratory.

As far as the authors are aware, no saprophytic plant with the form of a multicellular green alga has previously been reported. This plant is being placed in a family of its own, the Saprochaetaceae, near the Chaetophoraceae, and given the name of *Saprochaete saccharophila.*

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EXPLANATION OF PLATES

PLATE 22

Fig. 1. Habit sketch of a single plant. × about 18.

Fig. 2. End of branch showing several of the type of branch-tips used for vegetative multiplication. × 122.

Fig. 3. Single branch-tip after separation from parent plant. × 368.

Fig. 4. Outline of a few cells of a main axis showing position of branches. × 368.

Fig. 5. Outline of cell of a main axis from plant taken from a contaminated culture kept in the laboratory to show enlargement at ends of cells (drawing by Miss Alma Holland). × 335.

Fig. 6. Surface view of cell from plant killed in the fumes of osmic acid and stained with gentian violet showing the large nucleus and the appearance of the cytoplasm. × 368.

Figs. 7 & 8. Basal portion of older plants scraped from grass showing holdfast system that attaches plant. The ends of the holdfast branches have been broken off. Fig. 8, a, shows a young plant starting to grow upward from the holdfast branch of the parent plant. × 368.

Figs. 9–11. Early attachment stages of young plants on hard surfaces showing the flattening and branching of the tip cell. × 368.

Fig. 12. Stage showing the formation of a somewhat rhizoidal structure in the attachment of a branch-tip to a soft tender grass shoot, g. × 368.

Figs. 13 & 14. Young growing plants on tender grass blades. × 368.

Fig. 15. Branch from main axis of plant grown in maltose-peptone #5 solution showing peculiar enlargements at ends of cells. × 368.

Fig. 16. Tip of branch showing elongate thread-like growth of end cells which is very frequent either when growing in solution or on agar. × 368.

Fig. 17. Branch tip from maltose-peptone #5 solution showing threadlike elongation of one end cell and the breaking away of a small cell at the other end. × 368.

Fig. 18. Separating small cells from dividing branch-tips taken from maltose-peptone #5 solution. × 368.

PLATE 23

Fig. 19. Photograph of rocky stream bed showing thick mat of fluffy growth covering the rocks.

Fig. 20. Blades of grass with fluffy attached growth. × $\frac{5}{6}$.

Fig. 21. One month's growth in maltose-peptone #5 solution with considerable accumulation of fluffy growth on the bottom of the flask. × about $\frac{1}{3}$.

Fig. 22. Growth on agar one month after inoculation. Note accumulated surface growth in center of colony and zig-zag radiating strands. × $1\frac{1}{3}$.

Fig. 23. Termination of surface strand. Note bending and meandering type of growth and the large central axis in center with side branches running about parallel to this. × 92.

Fig. 24. Basal part of plant removed from blade of grass showing stubs of holdfast branches. × 67.

Fig. 25. Cell of a main axis from material kept in the laboratory for a few days. Note enlargements at ends of cells and the position of the origin of the side branches. Arrow points to nucleus with its large nucleolus. Unstained. × 475.

Fig. 26. End of branch that has grown down through the agar and is attempting to form a holdfast against the bottom of the petri dish. × 120.

PLATE 22

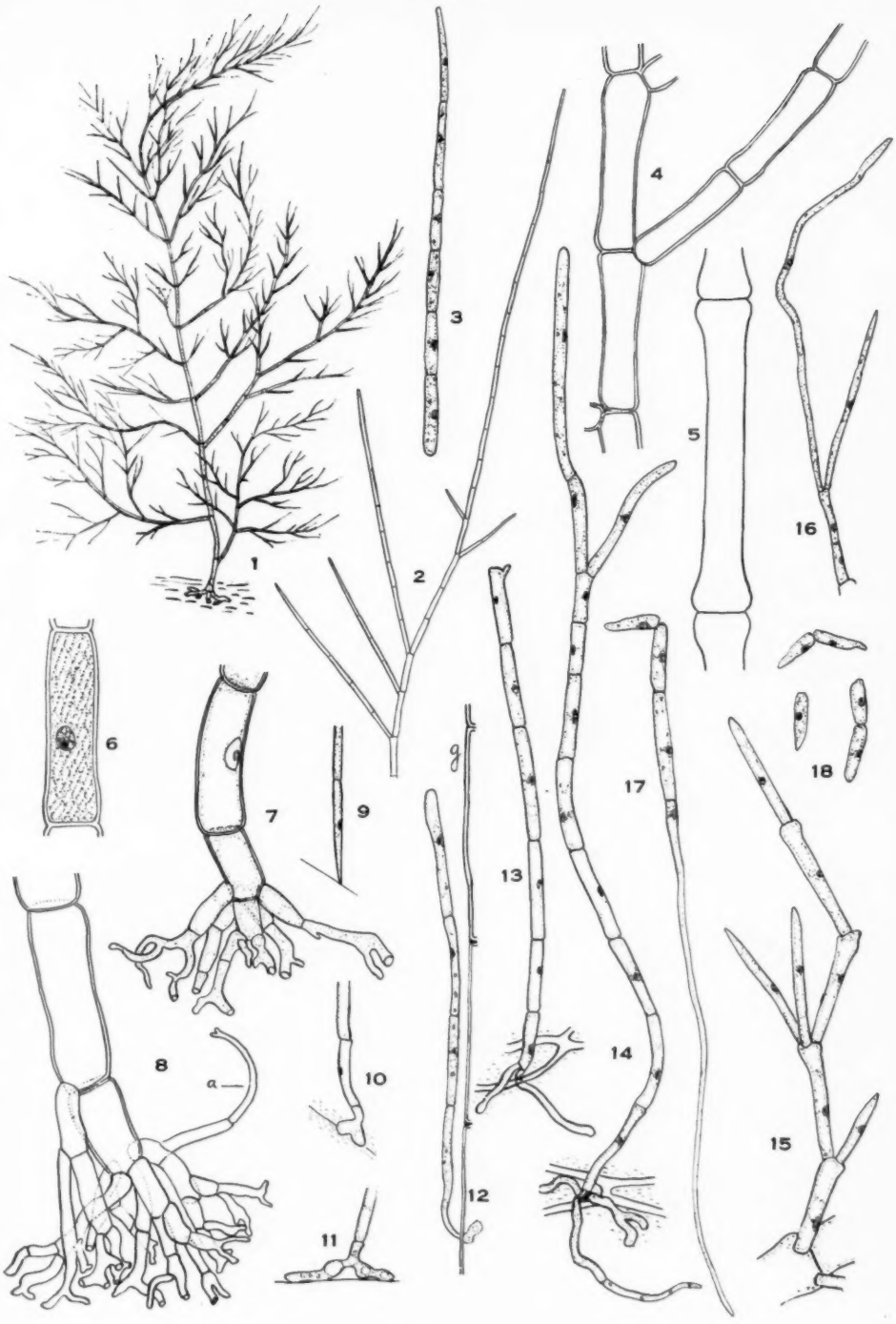
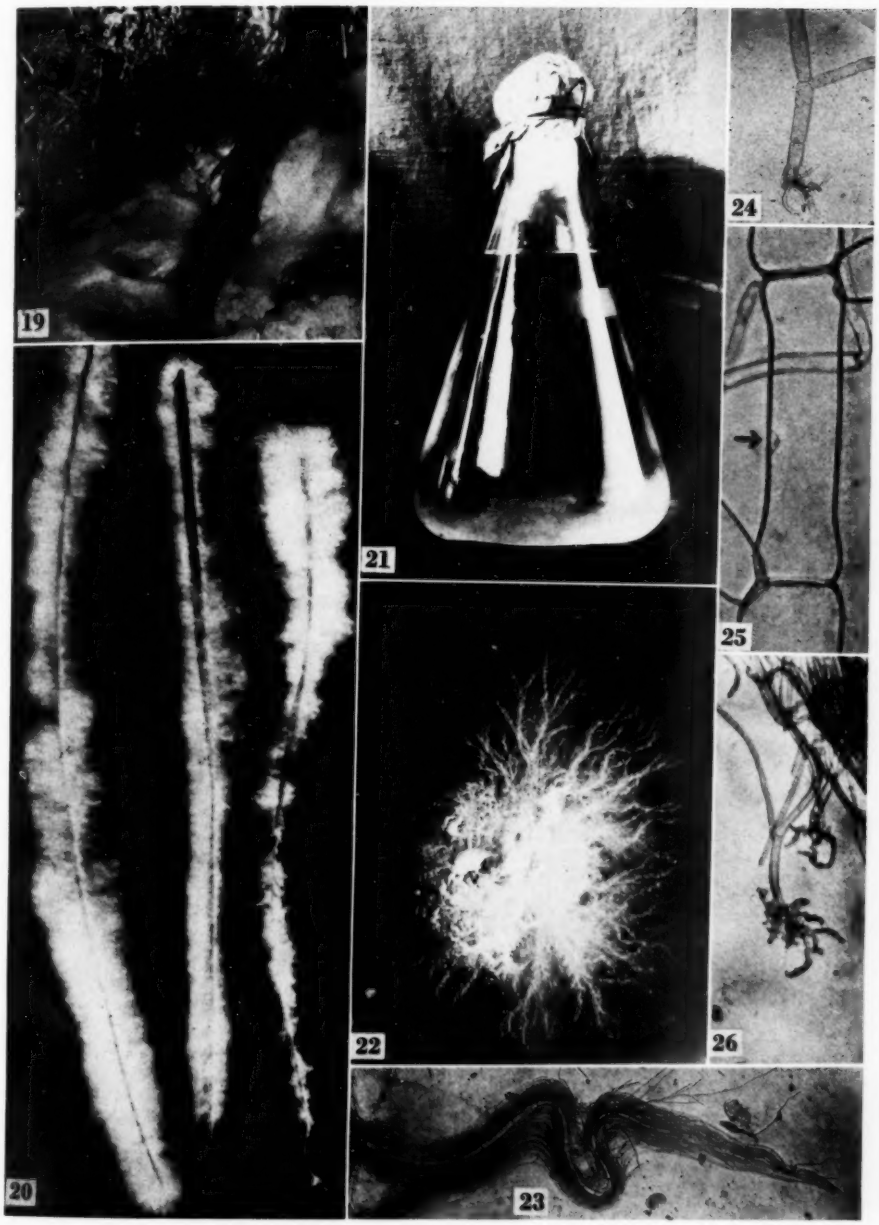


PLATE 23



STUDIES IN THE GENUS OLPIDIOPSIS. I

Resting Spore Germination in a New Species*

By Leland Shanor

Plate 24 and One Text Figure

Observations on resting spore germination in what is now considered to be an authentic species of *Olpidiopsis* apparently are not described in the literature. Fischer (1880, 1882) has described the germination of what he considered at that time to be the resting spores of *Olpidiopsis Saprolegniae*. The resting spores in this case, however, were not accompanied by the companion cell that is characteristic of this genus, so Fischer later (1892) decided that the plant that he had been working with before was a member of an entirely new and distinct genus for which he proposed the name *Pseudolpidium*. The chief difference in the two genera is the lack of a companion cell on the resting spores of *Pseudolpidium*.

Zopf (1884) has described rather briefly resting spore germination for his *Olpidiopsis Schenkiana*. Zopf's plant, however, possesses uniciliate zoospores rather than the biciliate type characteristic for *Olpidiopsis* and has been transferred to the genus *Pseudolpidiopsis* by von Minden (1915). Scherffel (1925) considers Zopf's observations of the number of cilia possessed by the zoospores of *O. Schenkiana* incorrect and reports this species to have biciliate zoospores. Tokunaga (1933,a) has since observed a form occurring on *Spirogyra* in Japan, considered to be *Pseudolpidiopsis Schenkiana* (Zopf) v. Minden, whose zoospores, he says, possess "a long cilium." There is a possibility, therefore, that

* This paper was presented before the Mycological Society of America, meeting at Richmond, Va., December, 1938, under the title "An Interesting Type of Resting Spore Germination in *Olpidiopsis*." The author wishes to express his sincere appreciation to Dr. W. C. Coker for his helpful suggestions during this study, and to Dr. J. N. Couch for carrying on the stock cultures during his absence from the University, June to September, 1938. This and the following paper are taken from material submitted to the Graduate Faculty of the University of North Carolina as a dissertation for the Ph.D. degree in the Department of Botany.

two very similar parasites occur on *Spirogyra* and that the observations on the number of cilia by all of these investigators is correct. Fitzpatrick (1930) follows von Minden in recognizing the correctness of separating the forms possessing uniciliate zoospores from those possessing biciliate zoospores.

Gäumann and Dodge (1928) cite Barrett (1912) as saying that the resting spores of *Olpidiopsis* germinate by zoospores which are discharged through an emission collar. Barrett (1912), however, states that as yet he had not observed cases of "oospore" germination in any of the species which he had studied, and gives, as the only cases in which it had been described up until that time, those by Fischer (1882) for *Olpidiopsis* (*Pseudolpidium*) and by Zopf (1884) for *Olpidiopsis Schenkiana* (now *Pseudolpidiopsis Schenkiana* (Zopf) v. Minden). In another species of *Olpidiopsis* occurring on an alga, *Olpidiopsis oedogoniorum* (de Wildeman) Scherffel, Scherffel (1925) states that resting spore germination is unknown.

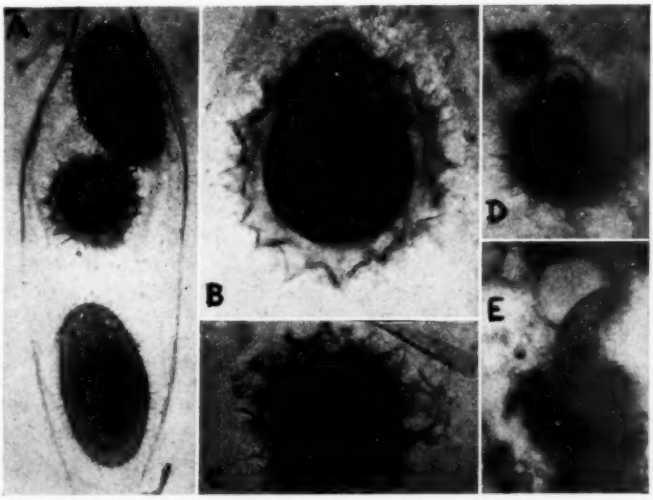
Gwynne-Vaughan and Barnes (1927), in summarizing the information known about *Olpidiopsis*, state that "Nothing is known about the fate of this resting structure, to which the empty antheridium remains firmly attached," but in a later edition (1937) suggest that "the oospore probably discharges biflagellate zoospores when it germinates." Smith (1938) states concerning this genus that "Germination of the zygote has not been observed." That the assumption of Gwynne-Vaughan and Barnes is correct is shown by the observations recorded in this paper.

MATERIAL STUDIED, INCLUDING THE DESCRIPTION OF A NEW SPECIES

The material from which these observations were made was collected from a roadside pool near Burgaw, North Carolina, March 18, 1938, parasitizing *Achlya flagellata* Coker. At this time the plant was carefully studied and resting spore germination looked for, but up until June no cases were observed in any of the cultures. At the close of the school term in June, new cultures were made and those were left with Dr. J. N. Couch to be kept going over the summer months. Upon my return in September, numerous empty resting spores with ruptured germ tubes and a number of others in the process of germination were found in the old cultures. The process was then carefully followed a number of times and the observations are given below.

This species of *Olpidiopsis* does not agree with any known species.

The companion cell to the resting spore is, in most cases, distinctly spiny, due to the fact that the spiny encrusting exospore wall that covers the resting spore is quite thick and also encloses the companion cell (Text figs. B. & C.). Some of the smallest resting spores may have smooth-walled or practically smooth-walled companion cells (fig. 12).



A. Enlargement of host hypha containing two typical spiny zoosporangia and one resting spore. × 160. B. Large resting spore before fertilization showing protoplasm in the antheridium. Spiny wall of the companion cell barely showing as it is partly masked by the disintegrating host protoplasm. × 340. C. Smaller resting spore in the dormant condition. Note the prominent spiny layer continued over the companion cell and outer dense and inner less dense regions of the resting spore protoplasm. × 340. D. Early stage in resting spore germination. Note hyaline tip of germination tube and clearer vacuolar region. × 250. E. Stage in germination just previous to zoospore formation. Note absence of vacuole and the presence of refractive globules in the now more or less homogeneous protoplasm; also shape of germination tube caused by conformity to the shape of the companion cell. × 250.

The manner in which the spiny layer is laid down is probably responsible for this variation, as there is often some variation in the sharpness of the spines of the exospore wall on the resting spores also.

Cornu (1872) has described the resting spores of *Olpidiopsis index* as having a spiny companion cell, but the spines on his plant are very

slender and are quite different from those of this fungus. The spines on the resting spores of this new species are rather broad at the base and are more like those possessed by *Olpidiopsis minor* Fischer. The companion cells of *O. minor*, however, are smooth rather than spiny. The resting spores of *Olpidiopsis minor* are also usually much smaller. Butler (1907), however, says that in the material studied by him they were larger, being about the same size as the resting spores of *O. Saprolegniae*. I have been fortunate enough to have cultures of *O. minor* for comparison and our plant seems to be too distinct to be even a variety of that species. The elliptical resting spores so frequently formed in cultures of *O. minor* have never been found in any cultures of this new fungus. In this plant resting spores are always spherical. Tokunaga (1933) has described a species from Japan, *Olpidiopsis spinosa*, in which both the resting spore and the companion cell are beset with long slender spines. Tokunaga's species is quite distinct in this respect. The average size of the resting spores of our new *Olpidiopsis* closely approaches that of *O. Saprolegniae* Cornu (not *O. Saprolegniae* (Cornu) Fischer), but the character of the spines on the two species is quite different and the companion cell of *O. Saprolegniae* is smooth-walled. The hyphal enlargement typically produced by *O. Saprolegniae* is also mostly terminal and frequently more spherical, whereas that of our plant is often intercalary and, when terminal, the enlargement is much more elongate. *Olpidiopsis index* Cornu and *O. spinosa* Tokunaga are the only other species known in which the companion cell is not typically smooth-walled.

Another very interesting variation from the usual situation reported for the genus is the presence of spines on the zoosporangia. The zoosporangia range from perfectly smooth-walled forms to ones densely covered with sharp spines (Text fig. A), but by far the greater majority have spines of some type. All imaginable intermediate gradations of spininess can often be found in one heavily infected hypha. The germination of both the smooth and spiny zoosporangia is identical and neither shows any more of a resting tendency than does the other. The spines on the zoosporangia are apparently of the same structure as those on the resting spores. The spines in neither case give a cellulose reaction with chloro-iodide of zinc, whereas the walls of both smooth and spiny zoosporangia are cellulose.

At first these spiny zoosporangia were suspected by the author to be the "Dauerspores" of Fischer's genus *Pseudolpidium*, but single spore isolation cultural studies have shown that they belong to this

Olpidiopsis. They certainly fit the descriptions of the resting spores of *Pseudolpidium*, particularly of *P. Saprolegniae* but sometimes of *P. fusiforme*, and appear remarkably similar to the figures found in the literature for this genus. Sparrow (1932) has reported that some zoosporangia of *O. minor* Fischer possess sharp scattered spines, but does not mention a resemblance to the resting spores of *Pseudolpidium*.

Because of the fact that there is so much variation in the spininess, not only of the sporangia but also of the resting spores, it might appear to one not studying this plant in detail that several species of the genus might be contained in a vigorous hyphal infection. This great variability makes the name *Olpidiopsis varians* seem appropriate. The description of this new fungus follows:

Olpidiopsis varians n. sp.

Zoosporangia single to many, formed either in terminal or intercalary swellings of the host hypha, elliptical, oval, or spherical, extremely variable in size from 60 by 40μ up to 350 by 140μ, frequently about 200 by 80μ, walls giving cellulose reaction with chloro-iodide of zinc, smooth to very spiny, spines slender and somewhat conical, up to 7μ long, not giving the cellulose reaction; exit tubes commonly one to three (as many as five have been observed). Zoospores oval to elongated, 3.8–4.6μ long by 2.3–3.0μ in diameter, usually about 4.2 by 2.8μ, biciliate, cilia of about equal length, measuring from about 4.2 to 4.6μ. Oogonia yellowish-brown and very variable in size, spherical, with usually one but sometimes two antheridia attached, 26 to 83μ in diameter (not including spines), averaging between 52 and 61μ; exospore wall colorless to yellowish, about 1.2μ thick, bearing usually coarse abruptly tapering spines which measure up to 8.6μ long and have a reticulum connecting them, not giving the cellulose reaction; endospore wall yellowish-brown, about 1.7μ thick, smooth, and giving the cellulose reaction. Antheridia (companion cells) usually spherical, 17 to 30μ in diameter, commonly about 26μ, wall occasionally smooth but typically covered by scattered spines similar to those on the oogonia but much shorter, 1.7μ at the longest, outer part of wall bearing spines usually colorless, inner wall having a slightly yellowish cast. Contents of antheridia pass into oogonia and antheridia on mature oogonia are empty. Germination takes place in this species by means of a germination tube which usually penetrates the companion cell. Biciliate zoospores are produced when the resting spore germinates.

Zoosporangiis solitariis vel numerosis, in cellulis terminalibus vel intercalaribus matricis formatis; ellipsoideis, ovalibus, vel sphaericis, valde variis, 60–350 x 40–140μ, saepissime 200 x 80μ; membrana, quae reactionem cellulosae dat, levi vel valde echinulata, spinis anguste conicis, longis ad 7μ, cellulosa carentibus; zoosporis ovalibus vel elon-

gatis, 3.8–4.6 x 2.3–3μ, saepissime 4.2 x 2.8μ, biciliatis, ciliis equalibus in longitudine, 4.2–4.6μ. Oogoniis luteo-brunneis, valde variis in magnitudine, sphaericis; antheridio uno vel duobus, 26–83μ in diam. (praeter spinas), saepissime 52–61; episporio hyalino vel luteo, 1.2μ crasso, spinis crassis acutis, longis ad 8.6μ, cellulosa carentibus; endosporio luteo-brunneo, 1.7μ crasso, levi, cellulosam habente. Antheridiis sphaericis, 17–30μ in diam., saepissime 26μ, levibus vel plerumque echinulatis, spinis longis ad 1.7μ.

Collected on *Achlya flagellata* Coker, from a roadside pool near Burgaw, N. C., March 18, 1938.

A detailed description of the germination of the zoosporangia of this species is unnecessary since the stages in maturation and germination are essentially the same as those described for other species by Barrett (1912), and for the zoosporangia of *Pseudolpidium Aphanomycis* by Butler (1907).

OBSERVATIONS ON MATURATION AND GERMINATION OF THE RESTING SPORES

After fertilization has taken place and the resting spore has formed an endospore wall about itself, the protoplast soon presents the mature structure. In the mature condition the resting spore has a very characteristic appearance. There is an outer region of very fine, densely granular protoplasm that is separated rather sharply from a central region of coarser granular protoplasm. Often small oil globules may be located in either region. In this condition the resting spore may lie dormant for an indefinite period.

The first noticeable change to occur indicating that the resting spore is going to germinate is the disappearance of the sharp dividing limitation between the outer dense, finely granular region and the inner more coarsely granular one. As this takes place, the denseness of the protoplasm in the outer region diminishes and soon all of the protoplasm appears similar except for the presence here and there of small oil globules. A large vacuole has now appeared and lies somewhat in an eccentric position. Unless conditions are changed, such as the addition of fresh water to a culture, resting spores may remain in this condition for a rather long period also.

Just before the germ tube is initiated, a slightly more hyaline region appears just under the companion cell (fig. 2). Before long the wall between the resting spore and the companion cell is dissolved away and the growth of the germination tube into the companion cell takes place

(fig. 3, & Text fig. D). This growth continues until the germination tube reaches the opposite wall of the companion cell. The germination tube commonly widens after reaching the opposite wall so that usually the width of the germination tube is equal to the width of the companion cell (fig. 7 & 8). This is not always the case, however, and sometimes there is a distinct space between the germination tube and the wall of the companion cell (fig. 4, etc.). The wall of the germination tube gives the typical reaction for cellulose with chloro-iodide of zinc. Usually a short time now elapses before the germination tube can dissolve its way through the opposite wall of the companion cell. In the meantime, the vacuole usually changes its shape continually, shifting its position occasionally, and often now more small oil globules can be seen.

After dissolving its way through the companion cell wall (fig. 4), the germination tube grows to a short distance beyond before ceasing to elongate and often at this point germination may again be arrested. This is particularly true when the material under observation is under a cover glass. When germination proceeds again, the protoplasm becomes more coarsely granular, small refractive globules appear scattered throughout, and the vacuole disappears entirely (fig. 7 & 8, and Text fig. E). In a short time, the initials of the zoospores can be seen (fig. 5) and, in a few seconds after their formation, the zoospores set up a motion within the resting spore. This shuffling, jerky movement continues until finally the end of the germ tube is ruptured after having become softened. When the rupturing takes place, the spores nearest the end of the germination tube are discharged so forcibly that they do not come to rest until some distance from the tip of the tube. Instead of swimming away immediately after the force of the discharge carries them no farther, they remain stationary for a second or two, as if bewildered, and then swim away rapidly. The other zoospores in the resting spore keep shuffling about and the ones nearest the opening escape as rapidly as the opening in the end of the germ tube will allow. The opening in the end of the germ tube is not much larger than will permit the escape of a single zoospore at a time. Usually within a few minutes all of the zoospores make their escape (fig. 6). These zoospores produced by the germination of the resting spores appear identical to those produced by the germination of the zoosporangia. They possess two cilia which are attached laterally nearest the anterior end of the spore and swim with one cilium directed forward and having the other one trailing behind (fig. 6 & 6a).

DISCUSSION

After learning the secret of the necessary elapse of time after fertilization, I have been able to get germination of the resting spores in my cultures almost at will. All that seems necessary to do is to place in fresh water on a slide the resting spores from a properly aged culture. Those resting spores that have already proceeded as far as the approximate stage shown by Fig. 2, will continue germination almost immediately. These may be covered with a cover glass provided sufficient water is kept on the preparation to keep the cover glass floating. Almost immediately, then, the hyaline area will appear under the companion cell and the dissolution of the wall between commences. The whole process from the condition shown by Fig. 2, until zoospores are released may take place within half an hour. In other instances, some resting spores may produce a germination tube and then remain in this condition for a day or two before completing zoospore formation. This is particularly true when a preparation for examination is placed in the same water in which the culture was growing and additional water is added from the same source, or when the cover glass is not continuously kept floating during the observation. The process of germination seems to be hastened by the continued addition of fresh water to the material being examined.

Zopf (1884) found that he could obtain the germination of the resting spores of his *O. Schenkiana* by allowing the resting spores to dry on a slide for a few days and then adding fresh water to these. I have tried this method with several species of *Olpidiopsis* but have not as yet been successful in obtaining germination by this method alone. I have also tried freezing resting spores after fertilization but this has likewise failed to hasten the maturation process in preparation for germination. The resting spores in a culture about three months old that had been dry for some time germinated soon after water was added. Germination seems to take place only after the elapse of time necessary for the maturation to be completed normally. I have obtained resting spore germination in only this one species, and as yet, none of the resting spores of any of the other species at hand have shown any signs of germination.

The explanation for the fact that the germination tube grows through the companion cell in this species may be that there is less resistance to its growth at this point. At other places the resting spore is covered with a thick encrusting exospore that probably offers considerable

resistance to the progress of the germination tube's growth. Even in the case of the smallest resting spores this is the rule, and it seems always to be the case in the germination of the larger ones. It is sometimes rather difficult to determine with certainty whether the germination tube has grown through the companion cell in cases where the tube is formed before the observations were begun, but this can usually be determined after careful observation. When two companion cells accompany a resting spore, germination seems to take place through only one of them (fig. 10). Cases where more than one germination tube have been formed in the germination of the resting spores have not been observed.

SUMMARY

1. A new species of *Olpidiopsis* is described as *O. varians* n. sp. Most of the zoosporangia of this species possess spines of some description, but some of the zoosporangia are smooth-walled. A resemblance of the spiny zoosporangia of this species to the "Dauerspores" of Fischer's genus *Pseudolpidium* is noted. The companion cell of this plant is also spiny in the majority of cases, but the species is quite distinct from the other two known species which possess this characteristic.

2. Germination of the resting spores of an authentic species of *Olpidiopsis* is described, apparently for the first time. A long dormant period after fertilization has been found necessary before germination will take place. In this species there is usually formed a germination tube which penetrates the companion cell and biciliate zoospores are liberated. These show no apparent difference from the zoospores released by zoosporangium germination.

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EXPLANATION OF PLATE 24

Fig. 1. Dormant condition of the resting spore. Protoplasm in two rather sharply defined regions, the outer one finely granular and dense, the inner one more coarsely granular and less dense. × 368.

Fig. 2. Early stage in germination. Protoplasm is more homogeneous and much less dense than in the dormant condition. Note hyaline region just under companion cell. × 368.

Fig. 3. Germ tube growing into companion cell. The hyaline tip is still quite prominent. × 368.

Fig. 4. Germ tube has dissolved its way through companion cell and now the protoplasm is becoming more coarsely granular. × 368.

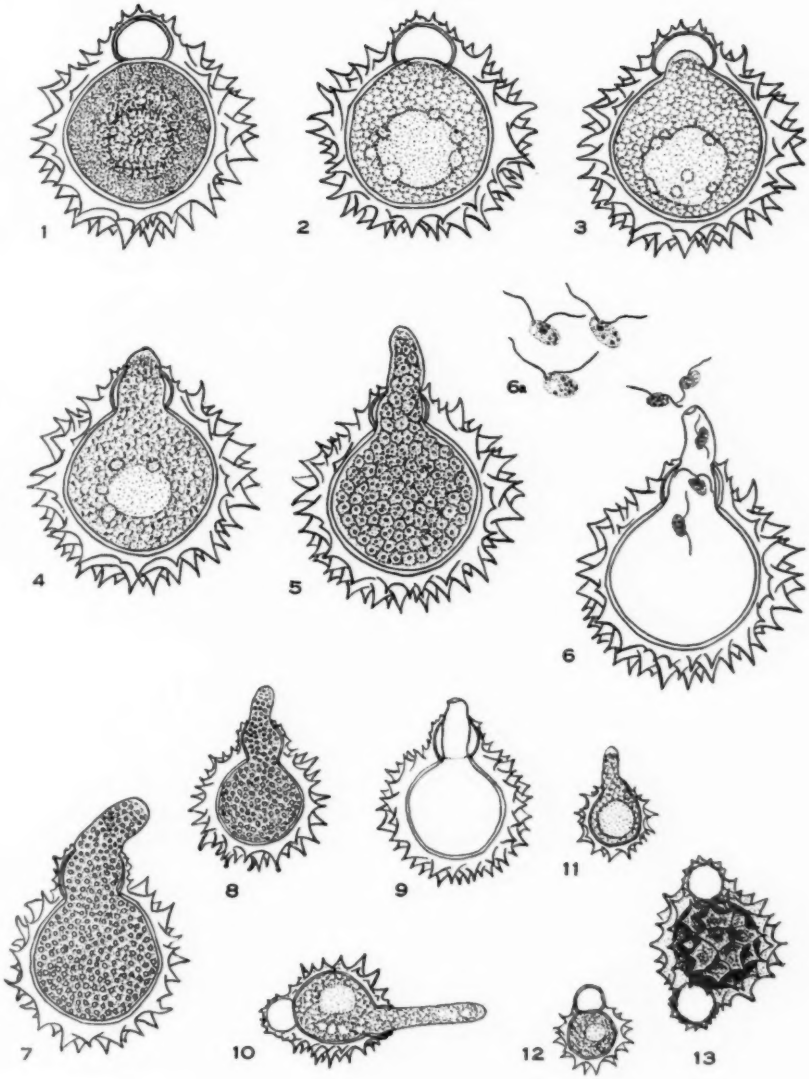
Fig. 5. Stage just previous to rupture of germination tube. Spores are almost completely formed and are in a very compact mass. Each spore contains one or more small refractive globules. × 368.

Fig. 6. Stage showing ruptured germ tube and escape of last zoospores. × 368. Fig. 6a, Zoospores killed in fumes of osmic acid and stained with gentian violet. × 1135.

Fig. 7. Stage intermediate between stages shown in Figs. 4 & 5. Protoplasm is more coarsely granular and numerous small refractive globules are present. Large vacuole is now absent. Basal part of germination tube completely fills companion cell. × 368.

Fig. 8. Smaller resting spore in same stage as shown in Fig. 7. × 368.
Fig. 9. Empty resting spore showing germination tube not completely filling the companion cell. × 368.
Fig. 10. Medium sized resting spore having two companion cells. Germination is taking place through only one of them. × 368.
Figs. 11 & 12. Two of the very smallest resting spores; one just before the formation of the germination tube, the other farther advanced. × 368.
Fig. 13. Surface view of resting spore accompanied by two companion cells showing ridges connecting spines. Surface view of the companion cells is not shown. × 368.

PLATE 24



UNIV. OF MICH.

STUDIES IN THE GENUS OLPIDIOPSIS. II

THE RELATIONSHIP OF *Pseudolpidium* FISCHER AND *Olpidiopsis* (CORNU) FISCHER*

By LELAND SHANOR

PLATE 25 AND 3 TEXT FIGURES

While collecting for parasites belonging to the genera *Pseudolpidium* Fischer and *Olpidiopsis* (Cornu) Fischer, the author became quite interested in the relationship between them, principally because they are so frequently collected together and because continuous culture has produced only *Olpidiopsis*. This interest was further intensified by the finding in the case of one species of *Olpidiopsis* just described in another paper, instances of clear gradation between what have been considered the zoosporangia of *Olpidiopsis* and what have been thought to be the spiny resting sporangia of *Pseudolpidium*.

In a perusal of the literature relating to the genus *Olpidiopsis* one immediately becomes impressed by the abundance of the citations where members of this genus are found frequently in association with members of the genus *Pseudolpidium*. It is also very interesting to note that the species of *Pseudolpidium* found occurring with a given species of *Olpidiopisis* remains relatively constant when it is collected, even though the stations where the collections have been made are widely separated geographically. It also seemed quite strange that any two parasites so similar in habits should be found occurring together over such a wide range. All of these facts indicated that some very interesting discoveries might be made if the problem were more thoroughly investigated. This study was begun in March, 1938, and has been continued to January, 1939.

Although these parasites were known before, Cornu (1872) was the

* The conclusions reached in this paper concerning *Pseudolpidium Saprolegniae* were briefly stated before the Mycological Society of America, meeting at Richmond, Va., December, 1938, in connection with the variation of sporangial forms of the new species discussed then and described in the first paper of this series. Mr. D. A. McLarty, at the same meeting, read a paper dealing primarily with the validity of *Pseudolpidium fusiforme*.

first to describe and name them as members of the genus *Olpidiopsis*. He observed the zoosporangia to be smooth-walled structures of varying sizes and shapes, and the resting spores to be spiny and thick-walled and to be accompanied by a usually smooth, thin-walled structure which he termed a "cellule adjacente." In one of his species, *Olpidiopsis Aphanomycis*, no resting spores were observed and in another, *O. incrassata*, the adjacent cell was not clearly determined.

In his early studies on *Olpidiopsis*, Fischer (1880, 1882) was unable to find an adjacent cell on the spiny structures which he thought were the resting spores of this genus and concluded that Cornu (1872) had been mistaken in his observations in this respect and that the resting bodies were not accompanied by an adjacent cell. When he later discovered the true resting spores of *Olpidiopsis* that were accompanied by the companion cell as had been described by Cornu (1872), Fischer (1892) concluded that Cornu was correct in his former observations. Fischer now retained the generic name given by Cornu (1872) for those forms whose resting spores were accompanied by the adjacent cell and established the genus *Pseudolpidium* to accommodate those forms which had been studied before in which the companion cell had been lacking. The chief difference in the two genera was, therefore, the presence of this cell on the resting spores of *Olpidiopsis* and the lack of it in *Pseudolpidium*. In other respects the two genera are alike and impossible to distinguish unless the resting spores are present.

In his new genus *Pseudolpidium*, Fischer (1892) placed six species. *Pseudolpidium Saprolegniae* was the name given to the plant studied previously by him and taken in part from Cornu's original *Olpidiopsis Saprolegniae*. *Pseudolpidium fusiforme* was the name applied to the plant which he had thought before was *O. fusiformis* Cornu, and was obtained by splitting Cornu's *O. fusiformis* to form this species of *Pseudolpidium* and *Olpidiopsis minor* n. sp. *Pseudolpidium Aphanomycis* was proposed doubtfully by transferring *O. Aphanomycis* Cornu, the species in which resting spores had not been observed, to this genus. *Pseudolpidium* (?) *incrassata* was also transferred doubtfully since the adjacent cell to the resting spore of *O. incrassata* Cornu had not been clearly shown. *Pseudolpidium glenodinianum* and *P. Sphaeritae*, in spite of the fact that their resting spores were unknown, were placed here by transfer from the genus *Olpidium* to which they had originally been assigned by Dangeard (1888, 1889).

Of the six species which he placed in the genus *Pseudolpidium*, Fischer had the opportunity to study only *P. Saprolegniae* and *P.*

fusiforme in any great detail. By this establishment of *Pseudolpidium* to include the forms lacking a companion cell on the resting spores, Fischer thought that the confusion caused by his previous work had been cleared up.

Those making a survey of the fungi of any certain region are not usually greatly concerned with culturing and studying in detail those forms that have been adequately described before, and so students as a rule accept an original authoritative description when making an identification unless some interesting observation leads to a more detailed study. Therefore, students since Fischer have been inclined to accept unquestionably the large spiny structures lacking a companion cell that are so frequently found associated with certain species of *Olpidiopsis* as the resting spores of *Pseudolpidium*, since the two were said to be commonly found together. Then, too, a determination has too frequently been made and reported as *Pseudolpidium* when only the large smooth-walled sporangia were seen.

MATERIALS AND METHODS

The isolates from which these observations have been made have been collected from various stations in North Carolina, mostly in the vicinity of Chapel Hill. They have all been obtained parasitizing watermolds that have grown on halves of boiled hemp seed which have been placed in water collections when these were brought into the laboratory.

The procedure followed for the isolation and purification of all cultures of *Olpidiopsis* and *Pseudolpidium* was as follows: When an original culture of the watermold was found to have hyphae infected with what appeared to be a species of either of these two genera of parasites, a hypha containing an early infection was carefully removed, washed, and then placed on M. P. #5 agar. In this way pure cultures of the host were easily and quickly obtained. Fresh pure cultures of the host for the isolation of the parasite were then secured by placing halves of sterilized hemp seed in a petri dish in water on small blocks of the agar containing the watermold. When the watermold hyphae began to appear around the edge of the hemp seed, infected hyphae bearing mature sporangia were removed from the original culture, cleaned thoroughly in distilled water, and then transferred to these young pure cultures of the host. Usually a vigorous infection by the parasite is soon obtained in this way. No attempt to get single spore or monosporangial cultures was made at this time, the purpose now being to get the parasite clean and growing vigorously on a single host plant.

As the study progressed and the relationship of smooth and spiny sporangia became more obvious, it was necessary to make monosporangial and single spore isolations. Monosporangial cultures were made in two ways: (1) A hypha bearing a single sporangium of the desired type was cut off from the culture, removed and thoroughly washed in distilled water, examined with the compound microscope for attached spores, and if found free of them, then transferred to a young pure culture of the host plant. (2) Single sporangia, when mature, were dissected out of the host hyphae by carefully splitting open the hyphal wall with a fine, sharp-pointed needle. These were then washed carefully in distilled water, examined under the compound microscope, and transferred by means of a pipette to a young pure culture of the host plant. This second method proved to be considerably more difficult to carry out than did the first, and, when it was determined that the results obtained by this second method were not different from the first, this one was discontinued in the later studies.

Single spore isolations were obtained in the following way: A hypha containing a mature sporangium of the desired type was cut off and removed from the original culture, thoroughly washed in distilled water, transferred to a drop of distilled water on another slide, and then placed under the high power of the binocular microscope so that germination could be followed. When the sporangium germinated, a few spores were drawn into a very fine-mouthed pipette as they emerged from the germ tube. These spores were then placed in another drop of distilled water and still more water added to this so that the spores would be allowed to scatter about more freely. After this a few spores were again drawn into the fine-mouthed pipette and transferred to a young pure culture of the host. It is frequently quite difficult to draw up a single spore to add to a culture of the host, but the number can be reduced to three or four. Even though more than one spore were added to a host culture, it is of no great importance, because spores added to a culture in this way proceed to isolate themselves when bringing about infection on the new culture. Each spore, when it brings about an infection on the host hypha, gives rise to a single thallus of the parasite which retains its identity throughout its development (on this observation see also Barrett, 1912), and, upon reaching its maximum growth, forms a single fruiting structure, usually a sporangium. Therefore, as each thallus from a single spore reached maturity and formed a sporangium, the hypha bearing it was carefully removed, washed, and then transferred to a fresh young culture of the host plant. In this way the progeny

of the spores from this sporangium all came from the infection brought about by a single isolated spore. Single spore isolations were made only to verify the results obtained from monosporangial cultures.

The water used in these cultural experiments was distilled water to which had been added a small amount of animal charcoal. This was then filtered and sterilized before being used.

A CONSIDERATION OF PSEUDOLPIDIUM SPECIES

(A) *Pseudolpidium Saprolegniae* (A. Braun) Fischer

The smooth and spiny sporangia of *Pseudolpidium Saprolegniae* described by Fischer (1892) as the zoosporangia and resting spores respectively, have been found not entirely confined to one single species of *Olpidiopsis*. The author has collected isolates producing both of these structures which have proven to be variations of sporangia of *Olpidiopsis Saprolegniae* Cornu, *O. vexans* Barrett (*O. Saprolegniae* (Cornu) Fischer), and *O. varians* Shanor. Since the smooth-walled zoosporangia of all of these species are much alike in shape and size, and since the shape of the spiny bodies of *Pseudolpidium Saprolegniae* has been described as not unlike that of the zoosporangia, when it was determined with certainty, this fact was not at all surprising. Although these *Pseudolpidium* types have appeared with all of the above mentioned species, detailed studies in this group have been largely confined to one *Olpidiopsis* species, *O. varians*. This species lends itself particularly well to a study of this kind because it quickly produces an abundance of sporangia, and in addition, has another advantage because its resting spores have been germinated. Then too, the spiny sporangia in the other species are much more rare and those of *O. vexans*, strictly speaking, have no spines but only wart-like deposits. Cultural studies with *O. Saprolegniae* Cornu and with *O. vexans* Barrett have been carried on only so far as to determine that the smooth and spiny-walled structures suggestive of *Pseudolpidium* were sporangial forms of these two species.

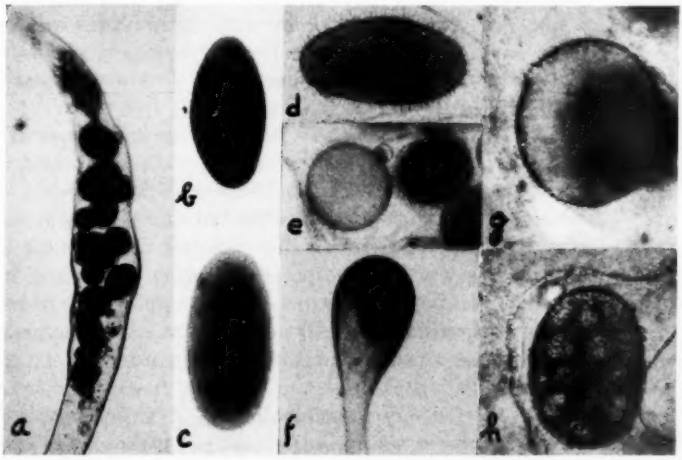
The first type of sporangia produced by the infection by spores from a monosporangial transfer of *O. varians* is usually of either the large smooth-walled or of the large spiny-walled *Pseudolpidium* type, these commonly appearing singly in a host hypha. In later infections in the same culture, brought about by the spores from the germination of these sporangia, zoosporangia regularly appear much smaller and many more of them are usually found in a single hyphal enlargement. As more sporangia mature in a culture, more spores are present to infect young

hyphae as they are produced by the host, and consequently the later increase in the number of sporangia in an enlargement is easily explained. In infections where a single spore has germinated on a hypha, a greater amount of host protoplasm has been available for the growth of the individual thallus than would be available for the growth of each where many spores have developed in a single hypha. This difference in the amount of available food for the growth in the two instances would explain the difference in the size of the sporangia under the two circumstances. Of course, some of the large sporangia are produced in older cultures along with the smaller ones, but this most frequently occurs where only a very few zoospores have brought about the infection of a hypha.

The length and abundance of the spines on the sporangia vary considerably. They may be small and sparsely scattered, long and scattered, or small or long and densely covering the sporangial wall. In still other cases the spines are reduced to mere warts on the wall and instances have been observed where this deposit was so slight as to be hardly observable. Treatment with chloro-iodide of zinc, however, makes the deposit plainly demonstrable because the sporangial wall itself gives a beautiful blue reaction with this solution while the deposit forming the spines remains colorless. All imaginable gradations from perfectly smooth-walled sporangia to those densely covered with long spines could often be found in a single heavily infected hypha from the progeny of a single spore isolation (Text fig. 1a; Plate 25, fig. 3).

The length of the spines or the abundance of them on any sporangium does not seem necessarily to be restricted to sporangia of any given size. Small sporangia may bear spines or be perfectly smooth-walled, and the same can be said of all sizes, even to the very largest ones. However, small sporangia are more commonly smooth-walled or have only a slight deposit, whereas large sporangia, although very often smooth-walled, more often bear longer and more numerous spines. This large spiny type of sporangium (Text fig. 1b, c, d; Plate 25, fig. 3) is the type that in all probability was misunderstood by Fischer (1892) for the "Dauerspore" when he established his *Pseudolpidium Saprolegniae.* Because sporangia of all types mostly appear singly when isolates are originally collected and also because of the difficulty with which the true resting spores of *Olpidiopsis* may sometimes be obtained (see Barrett, 1912, p. 219), it is not surprising that Fischer misinterpreted these structures as he apparently has done.

In my cultures not all of the spiny sporangia were more or less ellipsodial as is said to be typical for *Pseudolpidium Saprolegniae*, but some are somewhat fusiform and distinctly resemble some figures that have been given for *Pseudolpidium fusiforme* Fischer. The somewhat fusiform sporangia produced in cultures of *O. varians* are, however, generally



TEXT FIGURE 1. (a–e), *O. varians*. (a) Typical hyphal swelling caused by this species in which there are the spiny-walled sporangia of the *P. Saprolegniae* resting spore type, smooth-walled sporangia, and two *Olpidiopsis* resting spores. × 70. (b) *Pseudolpidium* spiny-walled resting spore type of sporangium seen in surface view. × 200. (c) Large elliptical spiny-walled sporangium of the type that is frequently figured as belonging to *P. fusiforme*. × 95. (d) Spiny sporangium, in median plane, showing the formation of the germination tube. × 160. (e) Empty spherical spiny type of sporangium showing germination tube and the associated smooth-walled cell still containing some protoplasm. × 115. (f & g), *O. Saprolegniae*. (f) Typical terminal infection of host hypha by this species. × 70. (A terminal infection swelling similar to this is also typical of *O. vexans*.) (g) Single empty spiny-walled sporangium showing the fine, short spines. × 270. (h) *O. vexans*. Sporangium showing few, irregularly scattered wart-like deposits found on some sporangia of this species. × 225.

larger, broader, and more elliptical, and cause much more of a swelling of the host hyphae than do those appearing alone in cultures of *O. fusiformis*. The spines on sporangia of *O. fusiformis* are also much straighter and more coarse (compare Text fig. 1d and Text fig. 2f).

Still other spiny zoosporangia may be perfectly spherical and be

covered with long slender spines. Often these large spherical sporangia have small, smooth, thin-walled cells associated with them and present the appearance of *Olpidiopsis* resting spores (Text fig. 1e). The protoplasm in these smooth-walled adjacent cells, however, does not pass over and fuse with that in the larger spiny one, but instead disintegrates so that often when the sporangium has germinated, some residue can still be seen in this accompanying cell. This spiny spherical type of sporangium just described is of particular interest because it shows more clearly the morphological relationship in the origin of the ordinary zoosporangia in *Olpidiopsis* to that of the resting spores.

The germination of the *Pseudolpidium* type spiny zoosporangia appears to be identical with that of the smooth-walled ones and the former seem to possess no more resting characteristics than do the latter. This is more clearly brought out when cultures heavily parasitized are allowed to dry. When this is done, the contents of both the smooth-walled and spiny-walled zoosporangia shrink away from the sporangial wall in an identical manner, and within a short time disintegrate, while the protoplasm in the true *Olpidiopsis varians* resting spores remains alive for a long time, and if sufficiently mature can be germinated.

Further proof that the spiny *Pseudolpidium* type resting spores are not resting spores but are, in reality, just sporangial variations of a true *Olpidiopsis* is brought out by the germination of the *Olpidiopsis* resting spores. If the resting spores of *Olpidiopsis varians* that are sufficiently aged are carefully drawn up into a pipette from an old culture and placed in a drop of fresh water, they will germinate almost immediately. If clean young cultures of the host are added to the fresh water in which the germinating resting spores are found, they soon become infected by the zoospores formed by the germination of these structures. This infection will, in time, produce all of the sporangial types of both *Olpidiopsis* and of *Pseudolpidium* and, in addition, the true resting spores of *Olpidiopsis varians*.

The large smooth and spiny-walled structures described as the zoosporangia and resting spores respectively of *P. Saprolegniae* do not therefore belong to a distinct plant, but are merely variations in the sporangial types of a good *Olpidiopsis*. They have appeared most abundantly in my cultures of *O. varians*, but when first observed and described by Fischer (1880, 1892) as belonging to *Pseudolpidium*, they were probably sporangial variations of *Olpidiopsis Saprolegniae* (A. Braun) Cornu. This is thought to be the case because the deposits referred to

as spines seen rarely on the sporangial walls of *O. vexans* Barrett are more wart-like and it is therefore unlikely that it was this species. The terminal hyphal swellings figured by Fischer (1880, Taf. X, fig. 5, 6, 7; 1882, Taf. XIII, figs. 2 & 3) containing the smooth and spiny-walled sporangia are the type produced typically by either *O. Saprolegniae* or by *O. vexans*, but the spines are of the *O. Saprolegniae* type.

Earlier Cienkowski (1855) and Pringsheim (1860) had figured both the smooth and spiny-walled sporangia which appear to be those of *O. Saprolegniae*, but neither author correctly understood the nature of the structures with which he was dealing. Pringsheim figured in addition a resting spore with the companion cell typical of *Olpidiopsis*.

(B) *Pseudolpidium fusiforme* (Cornu) Fischer

Of all the species of *Olpidiopsis* and *Pseudolpidium* that have been reported collected together, *O. minor* and *P. fusiforme* seem to appear the most frequently.

Cornu (1872) originally described the sporangia of his *Olpidiopsis fusiformis* as being most frequently fusiform or linear in shape and as being smooth-walled. The resting spores he described as having broad tapering spines and having a thin, smooth-walled companion cell.

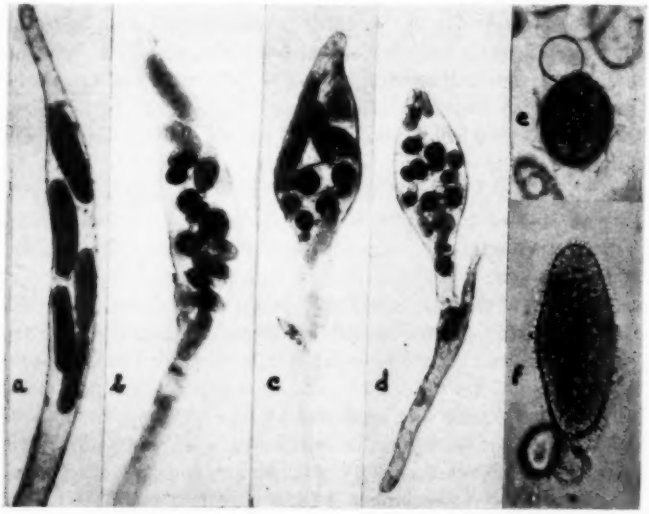
When Fischer (1880) first found the fusiform sporangia of this species, he also found fusiform spiny bodies and, since he did not find the resting bodies as had been described by Cornu, concluded that these must be the resting spores of *O. fusiformis*. After discovering a true member of the genus *Olpidiopsis* later, Fischer (1892) concluded that two plants were involved in Cornu's orignial description of *O. fusiformis*. The one of these Fischer described as having fusiform smooth-walled sporangia and fusiform spiny-walled resting spores, and named it *Pseudolpidium fusiforme* (Cornu) Fischer. This was the plant that he had observed earlier. The other plant Fischer characterized as having small, more spherical or oval smooth-walled sporangia and the type of resting spore as described by Cornu. For this plant Fischer proposed the name *O. minor*. He says, furthermore, that these two forms seem always to appear together and cites to substantiate this statement the observations of Cornu (1872) and of Reinsch (1878) also.

Butler (1907) has reported *O. minor* from India but in his plant the sporangia were described as being larger and similar to those of *P. Saprolegniae*, from which, he says, they could not be distinguished.

Petersen (1910) has obtained *O. minor* and *P. fusiforme* together from Denmark (Sealand and Jutland) but failed to find any of the *Pseudolpid-*

ium fusiforme spiny resting spores. Von Minden (1915) also states that *P. fusiforme* is often found with O. minor.

Sparrow (1932) has collected both *O. minor* and *P. fusiforme* from Cold Spring Harbor. He does not mention their ever appearing together in the same culture but says that some of the zoosporangia of



TEXT FIGURE 2. *Olpidiopsis fusiformis* Cornu. (a) Hyphal tip containing five sausage-shaped sporangia of the *Pseudolpidium fusiforme* type. × 70. (b) Hyphal tip containing in addition to the *P. fusiforme* type, small oval sporangia and resting spores typical of *O. minor*. × 70. (c) A hyphal enlargement showing fusiform sporangia of the *P. fusiforme* type and resting spores of *O. minor* type. × 70. (d) Swollen hyphal tip showing typical *O. minor* infection containing the small sporangia and resting spores. × 70. (e) A single *O. minor* oval type of resting spore showing the broad tapering spines and the smooth-walled companion cell. × 350. (f) Spiny-walled sporangium of the *Pseudolpidium* resting spore type showing scattered prominent spines. × 140.

O. minor possess spines. Tokunaga (1933) reports them both from Japan and found them frequently occurring together.

Matthews (1935) reports *P. fusiforme* and *O. minor* from Mountain Lake, Virginia, both growing on hyphae of the same collection of *Achlya flagellata*. They have appeared together invariably in my cultures, and the spiny-walled *Pseudolpidium* resting spore type were found almost as abundantly as the smooth-walled fusiform sporangia.

For the study to determine the relationship of this species, monosporangial and single spore isolations were made in the same manner as was done for the study and determination of *P. Saprolegniae*, and the results have been very similar. The first type of sporangia produced by the spores from a monosporangial transfer from any of the sporangial types are usually the fusiform ones, most commonly the smooth-walled type. If only a few spores infect a hyphal tip, very little if any enlargement takes place, the sporangia are sausage-shaped to fusiform, and if no further cultural studies were made, the immediate diagnosis would be *P. fusiforme* (Text fig. 2a). As these sporangia germinate, however, the infection brought about by these spores produced the typical more abundant, smaller sporangia and resting spores in the swollen hyphal tips characteristic of *O. minor* (Text fig. 2d). In a good many infections the fusiform sporangia, the small, more oval sporangia, and the resting spores of *O. minor* all appear together (Text fig. 2c). In other instances, the hyphal tip may be swollen and contain only fusiform sporangia and the *O. minor* type resting spores (Text fig. 2b).

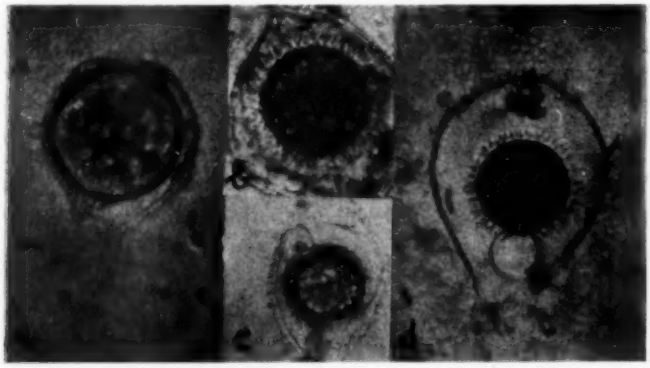
The resting spores of the isolates of *O. minor* which I have studied are more frequently oval than spherical but have the broad tapering spines characteristic of this species (Text fig. 2e). The spines on the spiny sporangia are not usually as numerous as are those on the spiny-walled sporangia discussed under the preceding species (Text fig. 2f). The interesting gradation between smooth-walled and spiny-walled sporangia is not so clearly evident as it was in the case of *O. varians*. Neither are all of the spiny sporangia fusiform or linear, but spiny sporangia may frequently be oval or nearly spherical as were those reported by Sparrow (1932). The very large spherical spiny sporangia with the functionless adjacent cell described under *O. varians* have not been observed in cultures of *O. minor*.

In the light of these observations, it becomes necessary to recombine *O. minor* Fischer and *P. fusiforme* (Cornu) Fischer under the original name give by Cornu, *Olpidiopsis fusiformis*, since what have been described as the smooth-walled sporangia and spiny-walled resting spores of *P. fusiforme* are merely sporangial variations of the same *Olpidiopsis*.

(C) *Pseudolpidium Aphanomycis* (Cornu) Fischer

The problem involved relating to the validity of *P. Aphanomycis*, as it has developed from my cultures, is not the same as that for the two species just discussed. In the former two, the *Pseudolpidium*

resting spores have turned out to be sporangial variations which had been misinterpreted originally. In the case of this species on *Aphanomyces*, the structures thought at first to be resting spores of *P. Aphanomycis*, upon careful examination, have proven to be inadvantageously oriented resting spores of *Olpidiopsis luxurians* Barrett (Text fig. 3, b & d; Plate 25, fig. 5, 5a). The hyphal enlargements produced by this parasite are usually in a different position on the hypha from those of the other species and are more spherical so that the resting spores are frequently found in such a position that the companion cell does not show. Therefore, at first, many of the resting spores appeared to lack



TEXT FIGURE 3. *Olpidiopsis luxurians*. (a) A terminal hyphal enlargement containing a single germinating sporangium. × 125. (b) Hyphal enlargement with one improperly oriented resting spore appearing as a resting spore of *P. Aphanomycis*. × 500. (c) Warted-walled resting spore of type described by Petersen for *O. Aphanomycis*. × 400. (d) Terminal hyphal enlargement containing a typical *O. luxurians* resting spore. × 400.

this structure and were suspected of belonging to *Pseudolpidium Aphanomycis*. It was only by dissecting these resting spores out, and by placing them in a drop of water so that they could be turned about, that the companion cell could be demonstrated in many instances.

The companion cell to the resting spores of this species on *Aphanomyces* is also so thin-walled that it frequently becomes collapsed in such a manner that its position is often difficult to determine among the spines on the resting spore.

The figure of the resting spore of *Olpidiopsis Alphanomycis* given by Dangeard (1891, Pl. 4, fig. 11) shows no companion cell and has been

considered to be a resting spore of *P. Aphanomycis* by Butler (1907). Butler himself found only one spiny structure in his cultures of *Pseudolpidium Aphanomycis*, but because it germinated so soon after it was formed, he questioned its being a resting spore and felt that what Dangeard had figured was likely the true resting spore of this species. After considerable observation on cultures of *O. luxurians* and a careful examination of Dangeard's figure, it seems likely that what Dangeard has shown was an *Olpidiopsis* resting spore whose companion cell was on the opposite side from that observed, or was a resting spore of *P. Pythii* Butler as is intimated by Minden (1915). Butler's spiny body was undoubtedly a spiny-walled zoosporangium, probably of this species also.

When Cornu (1872) named the species that he found on *Aphanomyces*, he was unable to find the resting spores. He was forced, then, to place this plant in the genus *Olpidiopsis* chiefly on the characteristics of the sporangia, since they were similar to those of the other species studied. Petersen (1910) described the resting spores of *O. Aphanomycis* as being similar to those of *O. Saprolegniae* (Cornu) Fischer, but as being somewhat smaller. This description was based on a single resting spore. Fischer (1892), in the meantime had transferred Cornu's plant doubtfully to *Pseudolpidium*. When Butler (1907) reported *P. Aphanomycis* from India, there was firmly established in the literature these two similar parasites occurring on *Aphanomyces*. Barrett (1912) added a second species to the genus *Olpidiopsis* which parasitizes *Aphanomyces* when he reported *O. luxurians*.

In my cultures of *O. luxurians*, the spines on the resting spores are in some cases reduced to such an extent that they are merely warts, and so might very easily fit Petersen's description of the resting spores of *O. Aphanomycis* (Text fig. 3c). Since this is the case, and if both Petersen and Barrett were dealing with the same plant, it is unfortunate that the single resting spore found by Petersen should have been the uncommon warty type. It is, therefore, quite possible that Cornu (1872), Dangeard (1891), Butler (1907), Petersen (1910), and Barrett (1912) were all working with the same species, and if this be true, it should be considered under the original name given by Cornu, even though the description which he gave was incomplete. Whether this possibility should prove correct may be cleared up by the collection and study of a larger number of isolates of *Olpidiopsis* occurring on *Aphanomyces*.

DISCUSSION

The relationship between certain members of the genus *Pseudolpidium* and members of the genus *Olpidiopsis* is clearly brought out in the foregoing cultural studies. However there still remain several species of the genus *Pseudolpidium* that have not been treated, species that so far have not been collected here. Nevertheless, it would seem in order to review what is known about these and to consider their status since the validity of the genus *Pseudolpidium* is very questionable.

When Fischer (1892) transferred *P. glenodinianum* and *P. Sphaeritae* to *Pseudolpidium* from *Olpidium*, the resting spores were unknown, and the change was based on the characteristics of the sporangia and the zoospores. Since the resting spores are the diagnostic structures separating *Olpidiopsis* and *Pseudolpidium*, these two species might quite possibly be good members of the genus *Olpidiopsis* when their resting spores are discovered. Von Minden (1915) more correctly treats these two species when he includes them questionably in the genus *Pseudolpidium*, placing in this same category *P.* (?) *deformans* Serbinow. It would seem even more correct now to consider these species questionably as belonging to the genus *Olpidiopsis* rather than to *Pseudolpidium*.

Fischer's transfer of *Olpidiopsis incrassata* Cornu to the genus *Pseudolpidium*, even though done doubtfully, seems unjustified, for this species should in all probability remain in the genus *Olpidiopsis* where originally placed by Cornu. Even though Cornu (1872) could not demonstrate clearly a companion cell in all cases, his fig. 12a, shows what seems most certainly to be a companion cell to the resting spore. The structure of the resting spores also indicates a species of *Olpidiopsis*.

It is, therefore, evident that none of the species placed in the genus *Pseudolpidium* by Fischer (1892) can remain there unquestionably. Some must be combined with certain species of *Olpidiopsis*, and those whose resting spores are unknown probably belong in this genus also.

Butler (1907) has described two species of *Pseudolpidium* found parasitizing *Pythium*, *P. Pythii* and *P. gracile*, whose characteristics answer those originally given for this genus. Butler reports that a starved resting spore sometimes persists beside a vigorous spiny one in *P. Pythii* so that these structures simulate the companion cells found in *Olpidiopsis*. He clearly points out the fate of these, showing that they do not function as antheridia as do the companion cells of *Olpidiopsis*. It is hoped that both of these species may be collected soon for they need more critical study. Another species, *P. stellatum* described by Sawada, and translated from the Japanese by Tokunaga (1933), also needs to be

studied more critically before its systematic position can be accepted with certainty.

Of all the species that have been placed in the genus *Pseudolpidium*, the ones that seem the most valid are those described by Butler (1907). Although some combinations of species of *Pseudolpidium* and *Olpidiopsis* have been made in this paper, and a reconsideration of others is suggested, a redescription of the genus *Olpidiopsis* to include the *Pseudolpidium* resting spore type of sporangia will be left to another paper now in preparation, which treats the genus *Olpidiopsis* taxonomically.

SUMMARY

In a monosporangial and single spore isolation study of collections originally appearing to be species of *Pseudolpidium* and *Olpidiopsis*, it has been found that all isolates appearing as *Pseudolpidium* have, by continued cultural study, turned out to be *Olpidiopsis*. The spiny resting spores described for *Pseudolpidium* have been found to be either the spiny zoosporangia or misinterpreted resting spores of the various *Olpidiopsis* species as follows:

a) *Pseudolpidium Saprolegniae* resting spore types have been found occurring abundantly in monosporangial cultures of *O. varians*, and frequently in cultures of *O. Saprolegniae* Cornu, the species to which these *Pseudolpidium* structures likely belonged when described. What might loosely be termed spines on the sporangial walls of *O. vexans* Barrett are merely wart-like deposits. *Pseudolpidium Saprolegniae* is, therefore, not a specific fungus but a combination of sporangial forms of authentic species of *Olpidiopsis*.

b) *Pseudolpidium fusiforme* zoosporangial and resting spore types are only sporangial variations that appear in cultures of *Olpidiopsis fusiformis* Cornu. *Olpidiopsis minor* Fischer is likewise not a distinct species, and must be recombined with *P. fusiforme* under the original name assigned by Cornu.

c) *Pseudolpidium Aphanomycis* resting spores appear to be only inadvantageously oriented *Olpidiopsis luxurians* resting spores or those whose companion cell has collapsed in such a manner that it is difficult to be detected among the spines of the resting spore. The question is also raised as to whether more than one species of *Olpidiopsis* occurs on *Aphanomyces* or if *O. Aphanomycis* and *O. luxurians* are not the same species.

The remaining *Pseudolpidium* species are reviewed and their status discussed. None of the species originally placed in the genus when it

was established can remain unquestionably as *Pseudolpidium* species. The continuance of the genus seems to rest on only two species, *P. Pythii* and *P. gracile*, both described by Butler. Even these two species need further critical study.

A redescription of the genus *Olpidiopsis* to include the spiny sporangial types described as resting spores of certain species of *Pseudolpidium* is necessary, but is being left to another paper.

In conclusion the writer wishes to express his sincere appreciation to Dr. W. C. Coker, under whom this study has been undertaken, for his helpful suggestions and interest in the problem, and to Dr. J. N. Couch for his advice and suggestions on certain phases of the investigation.

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EXPLANATION OF PLATE 25

Fig. 1. *O. vexans.* Terminal enlargement of hypha of *Saprolegnia litoralis* containing one resting spore; three smooth-walled sporangia, two of which are germinating; and two sporangia on whose walls are irregularly scattered wart-like deposits, one empty, and the other not yet beginning to germinate. × 123.

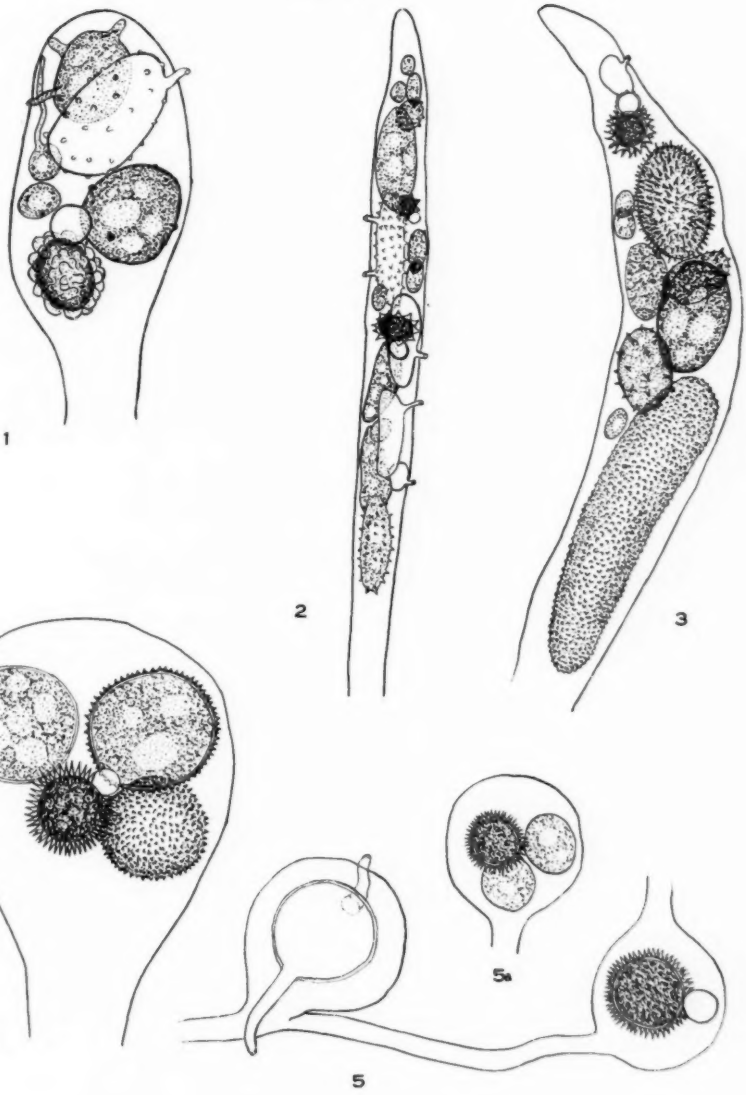
Fig. 2. *O. fusiformis.* Hyphal tip infection in *Achlya imperfecta* showing smooth and spiny-walled fusiform sporangia of the *Pseudolpidium fusiforme* type; small oval smooth and spiny-walled sporangia; and two resting spores. × 123.

Fig. 3. *O. varians.* Terminal swelling of *Achlya flagellata* hypha showing smooth-walled sporangia of various sizes; two sporangia with scattered sharp spines; one very densely spiny sporangium; one very large sporangium whose spines are reduced to small wart-like deposits; and one resting spore. × 123.

Fig. 4. *O. Saprolegniae.* Terminal enlargement of hypha of *Saprolegnia ferax* showing one smooth-walled sporangium; two spiny-walled *Pseudolpidium* resting spore type sporangia, one in median plane and the other in surface view; and one resting spore of this species. × 123.

FIG. 5. *O. luxurians.* Two intercalary swellings of a hypha of *Aphanomyces laevis,* one containing an empty sporangium and the other a typical resting spore of this species. 5a. A terminal swelling of an *Aphanomyces* hypha containing two sporangia and one resting spore. The resting spore is oriented to give the appearance of being a *Pseudolpidium* resting spore since companion cell is on opposite side. × 175.

PLATE 25



NEW MITES FROM WESTERN NORTH CAROLINA

By Arthur Paul Jacot

Plate 26

The following species were obtained from litter samples taken from the Bent Creek Experimental Forest (ten miles south of Asheville on the Brevard road), which were taken to endeavor to determine the extent and rôle of the fauna of litter in causing litter reduction. The types are to be deposited in the U. S. National Museum. Figures of species not here illustrated are in my files and are to appear in a more comprehensive publication.

Cultroribula divergens sp. nov.

Distal end of lamellae directed laterad; anterior edge of rostrum drawn downward in two attenuate cusps with the area between them (as seen in dorsal aspect) thinly sclerotized; anal and genital apertures close together; abdomen quite rotund; tectopedia I well developed, forming a bulge in dorsal outline of sides of cephaloprothorax; size varying in length from 0.23 to 0.24 mm., in breadth from 0.16 to 0.168 mm.

Cotypes: One hundred seven specimens from Andropogon field' herb-moss interspaces between grass plants, Case Place, Bent Creek Exp. Forest; taken February 6th, slide 34F24-8.

No individuals were obtained from the grass clumps.

Cultroribula trifurcatus sp. nov.

Distal end of lamellae directed anteriad though slightly more distant than further back; anterior edge of rostrum drawn forward in three attenuate cusps (trifurcate) as seen in dorso/ventral aspects; anal and genital apertures distant more than half length of genital aperture; abdomen somewhat flattened posterolaterally but quite broad; tectopedia I not forming a bulge in dorsal outline of sides of cephaloprothorax; size smaller and more slender: total length 0.214 to 0.224 mm., breadth 0.128 to 0.14 mm.

Cotypes: four specimens from dogwood (Cornus) litter, thirty-year-old field south of bunk-house, Bent Creek Exp. Forest; taken September 20th, slide 34F4.3-54.

Oppia elongata

Oppia elongata 4, p. 11, I rename *Oppia parviaures* **nom. nov.** since *Dameosoma elogantum* corrected on plate legends to *D. elongatum* 6, p. 43, is an *Oppia*.

Oribotritia virginiensis 3, p. 257, pl. 42

Original figure 77 is correct except that the interrogation points on the aspis should be changed to minute circles to represent the rostral and lamellar bristle insertions. This posterior and lateral position of the rostral bristles is most unusual. In lateral aspect the pseudostigmatic organs look like a black spot because they are viewed end on. The fine combing of the notogaster makes the animal appear iridescent in life and in alcohol. The pocking is faint and appears to be on the inside of the body wall.

I have several specimens from various litter samples from the Bent Creek Exp. Forest.

Euphthiracarus pulchrus pisgahi subsp. nov.

Differs from the species (3, p. 250, pl. 39, fig. 59) in having pseudostigmatic organs somewhat wider but with almost smooth head; anogenital plates pocked over their entire surface.

Cotypes: Six specimens from oak litter, east slope of ridge above Poplar Cove; Bent Creek Exp. Forest; taken July 15th, slide 35F6.2-37.

Phthiracarus apiculatus sp. nov.

Aspis with rostrum projecting beyond rim; carina absent; rostral bristles not unusual; vertex bristles not evident; pseudostigmatic organs oval, eyed, distal end drawn out into a hyaline, sharply pointed, downcurved apicule which is nearly as long as body of organ; notogaster oval, not high, tan to greenish grey (in small specimens), bristles fine, below medium length, depressed, angled proximad of middle, a1 usually near edge of collar; no accessory plate spoon; ventral plate with small, well spaced denticles; genital covers with anterior edge only slightly swollen; anal cover bristles II:1 to II:3 not visible in lateral aspect, I:1 and I:2 widely spaced, I:2 much longer than I:1; diagonal length of notogaster 0.37 to 0.58 mm.

Cotypes: Forty-eight specimens from oak litter, old growth stand, compartment 20, along brook, Bent Creek Exp. Forest; taken August 12th, slide 35F8.2-74.

Phthiracarus setanus sp. nov.

Aspis with very slender, nonprojecting rim; carina absent; rostral bristles not unusual; vertex bristles springing from a definite swelling, arched, just visible in lateral aspect; pseudostigmatic organs long, rodlike, pointed like a lead pencil, bent near pseudostigmata, distal third slightly more swollen than proximal two-thirds; notogaster oval, not high, bristles rather long, the longest about equal to length of genital covers, a1 near edge of collar; ventral plate denticles small; accessory plate without spoon; anterior edge of genital covers barely swollen, the bristles quite conspicuous; anal cover bristles I:1 and I:2 much shorter than II:1 to 00:3, II:2 inserted near transverse plane passing through I:2, II:3 distant from median edge of cover; diagonal length of notogaster 0.5 to 0.65 mm.

Cotypes: Eight specimens from litter of laurel slick, north side of Shut-in-Ridge, east of Walnut Cove, Bent Creek Exp. Forest; taken May 8th, slide 35F1.4-31.

Somewhat resembling *Ph. boresetosus* but bristles shorter; with ventral plate denticles; length of anal cover bristles the reverse; no accessory plate horn.

COCCORCHESTES 5, p. 573

Paul H. Oehser, editor of the U. S. National Museum, has kindly called my attention to *Coccorchestes* T. Thorell 1881 (Ann. Mus. Genova, vol. 17, p. 671, Araneina). I therefore propose as substitute for the generic name of the mite, *Coccorchestes humicolus*, the generic name *Oehserchestes* **nom. nov.**, making *Oehserchestes humicolus* **comb. nov.**

Histiostoma decemvirgae sp. nov.

Abdomen considerably broader and higher than cephaloprothorax; dorsal face with four pairs of conical apophyses each bearing a long, stout, backward curved bristle the length of which is nearly equal to breadth of abdomen, cephaloprothorax with one pair; ventral suckers broad-ovate; tarsi long and slender, tibiae longer than broad (tibiae I twice as long as broad); genuals one and a half times length of tibiae; tibiae with dorsal bristle long and stout, as long as dorsal bristles; sensory club inserted on proximal edge of tarsus, depressed, close to face of tarsus; ventral face with three of the usual spines, one at distal end, one slightly distad of distal fourth, and one at center; hind tarsi longer than tarsi I, bristles similar but with dorsal face bristle at proximal instead of distal fourth; mandibles acicular, palp bristles rather short; total length of adult females 0.2 mm.

Cotypes: Two specimens from litter of old woodland of a thirty-five degree, south slope, Bent Creek Exp. Forest; taken June 17th, slides 35F4.2-1a and -31.

The ten long, stout dorsal bristles, standing high above the body, make this the most easily recognized described American Histiostoma.

Histiostoma tesselata sp. nov.

Figures 1 and 2

Abdomen depressed, posterior end bluntly tapering, sides with flat winglike expanses overhanging the legs, middle area of dorsum areolated, bristles very fine, straight; posterior ventral suckers short, oval, small end anteriad; mouth parts directed forward; rostrum broad, truncate in dorsal aspect; palps large transverse, their bases encompassing the slender mandibles; anterior bristles of palps long, directed upward, posterior bristle quite short; mandibular bristle short, inconspicuous; tarsi I not tapered or constricted, sensory club small, inserted at proximolateral edge of tarsus, not noticeable in lateral aspect; total length of body (including palps) 0.26 mm., of abdomen 0.15 mm., breadth 0.11 mm.

Cotypes: Three specimens from rift of oak litter, 200-year-old woods, Shut-in-Ridge (station 17, compartment 5), Bent Creek Exp. Forest; taken June 11th, slide 35F3X-9.

This lot also included two other specimens on slides 35F3X-7 and -18.

Histiostoma verrucogenicula sp. nov.

Figures 6 to 9

Abdomen broad, rectangular, with an irregular, median hump, the bristles short, bent, depressed; cephaloprothorax much narrower than abdomen, anterior end truncate; mouth parts broad, directed downward so as to be barely visible from above; mandibles acicular, bristle short; anterior bristle of palps long, stout, extending ventrolaterally well beyond legs I; posterior bristle of palps short, strongly recurved; ventral suckers elongate-ovate (quite slender), extending mesad of legs III and IV, the broad end posteriad; leg bristles well developed, sensory club appears to be inserted in a notch cut out of dorsolateral edge of tibiae, thus appearing to spring from distal end of tibiae in lateral aspect; dorsoproximal end of tarsi I with a large wart (excrescence) composed of four or five transverse, crowded ridges; total length of body 0.34 mm., of abdomen 0.2 mm., breadth 0.18 mm.

Cotypes: Four specimens from mull litter of Rocky Cove, Bent Creek Exp. Forest; taken September 8th, 1934, slide 34F1-4.

Two immatures (?) from Liriodendron litter of a south cove, Shut-in-Ridge (B.C.E.F.); taken June 17th, slides 35F4 X-37, 35F4.2-28.

Histiostoma verruca sp. nov.

Figures 4 and 5

Length of females 0.25 to 0.29 mm., of males 0.18 to 0.2 mm.; skin granular; body oblong, with posterior bulge, dorsoposterior face of abdomen with a fairly large excrescence, oval in dorsal aspect; bristles short, nearly straight, tapering rapidly; ventral suckers elongate, one between legs II and III, one mesad of legs IV, parallel to median plane; sensory club inserted on posterior edge of tarsus; leg bristles short, except distal which is slightly over half the length of tarsus; mandibles needlelike (unhooked), untoothed, bristle long, curving well beyond sides of rostrum; palp bristles long, fairly stout; males with posterior end of abdomen produced as two prominent, clear edged, semicircular lobes (recalling Histiogaster when seen in dorso-ventral aspects) the posterior edge of which bears a minute bristle inserted laterad of center of lobe; the four suckers close to median plane, oval, the anterior pair separated from the posterior pair by their own length.

Cotypes: Two females from litter of old woodland of a thirty-five degree, south slope, Bent Creek Exp. Forest; taken June 17th, slide 35F4.1-2.

Most closely related to the European *H. fimetarius* as figured by Berlese (1, fasc. 9:7), but easily recognized by the rather large median pea-wart on posterior end of its back.

Although the commonest litter Histiostoma of this region it is uncommon. With the cotypes (but mounted on different slides) were fourteen additional females and three males. Another lot from a northern cove (Rocky Cove), contained three females and one male.

Macrocheles (Coprholaspis) appalachicus sp. nov.

Bristles of dorsum numerous, fine, overlapping, more numerous behind, no particular shoulder bristles; hypostome with a prominent, stout median spine, anterior edge finely sawtoothed to smooth; sternal, genital and anoventral scutes faintly, coarsely areolate, the areolations on sternal scute usually forming concentric semiovals culminating in the "linea oblique anteriores," the anterior ends of which are as approximate as they are distant from the ingular bristles; ingular and metasternal scutes fused to sternal; epigynal scute broad; anoventral scute in males fused to sternal and to peritremal, in females distinct, extending laterad as far as does the peritremal; no repugnatorial pores; no porose (punctate) areas; sculpturing of anogenital scute resembling fish scales; tarsi I retain a diminutive ambulacrum with the usual two nails, half the length of terminal bristles; tarsi II of females with seven stout spinelike bristles (five ventral, two dorsal); distal end of horn of male femur II three-ridged; femora IV unarmed; proximoventral edge of tarsi, tibiae and genuals denticulate; size variable: total length of dorsal shield of females 1.3 to 1.65 mm., breadth 0.8 to 1.0 mm.

Cotypes: Two males and a female from deciduous litter west side of ridge above Poplar Cove, Bent Creek Exp. Forest; taken July 29th, slide 35F7.1-25.

Most closely related to *M. bihastatus* 2, p. 286, fig. 1, differing in absence of the two lateral teeth, in absence of shoulder bristles, the greater armature of female tarsus, and the much greater size.

Twenty-four other adults from ten lots of forest litter of various types about the Bent Creek area are before me, never more than four per square foot. This species may thus be regarded as thinly sprinkled about the forest floor of this region.

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PLATE 26

Histiostoma tesselata sp. nov.

Fig. 1. Lateral aspect of cephalon and leg I; ×500±
Fig. 2. Dorsal aspect of cephalon; ×500±
Fig. 3. Dorsal aspect of abdomen (sketch); ×318±

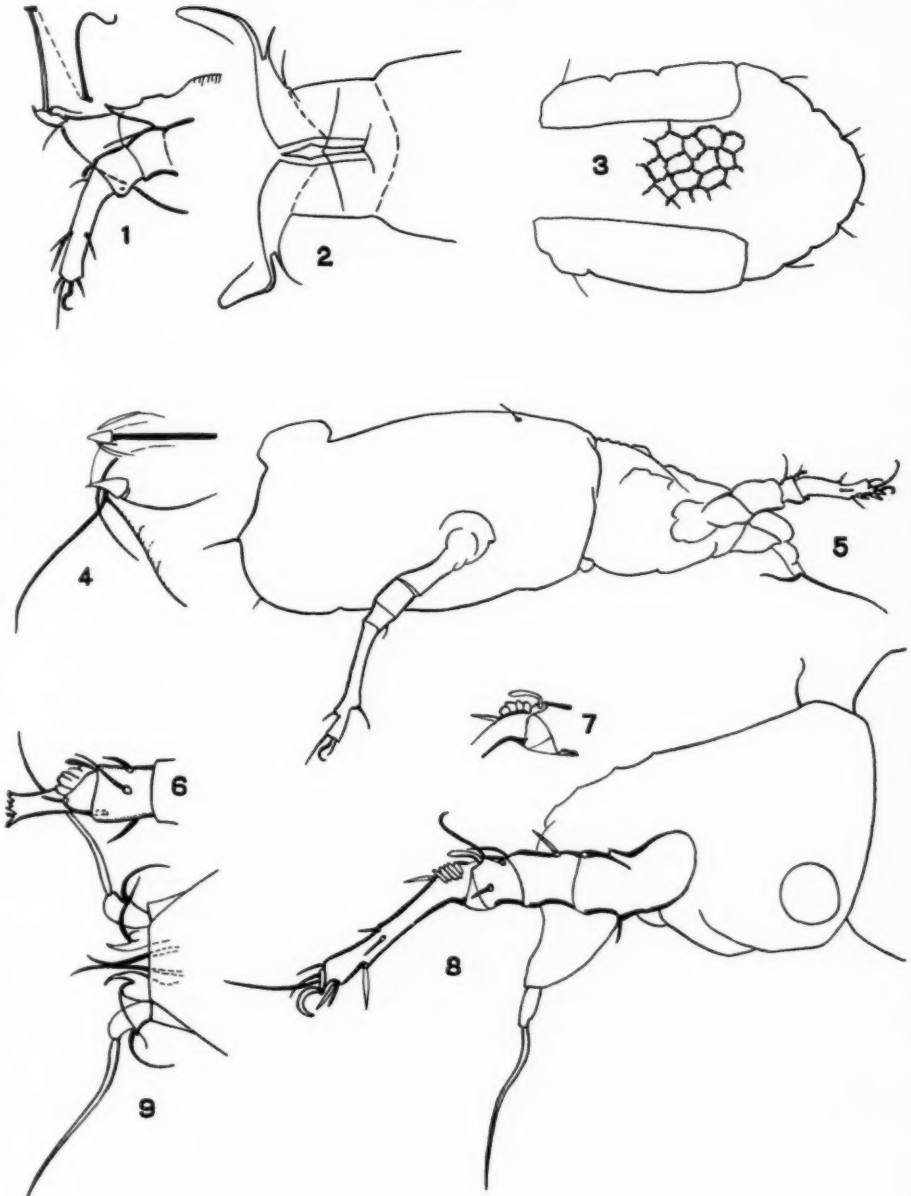
Histiostoma verruca sp. nov.

Fig. 4. Ventral aspect of mouth parts (right half); ×668±
Fig. 5. Lateral aspect, conspicuous bristles only; ×318±

Histiostoma verrucogenicula sp. nov.

Fig. 6. Dorsal aspect of right tibia I, showing wart and clavate bristle; ×500±
Fig. 7. Mesal aspect of same; same mag.
Fig. 8. Lateral aspect of cephalon and leg I; ×500±
Fig. 9. Dorsal aspect of mouth parts; ×500±

PLATE 26



THE ADMINISTRATION OF LARGE DOSES OF ASCORBIC ACID AND OF METHIONINE TO THE CYSTINURIC*

By James C. Andrews and Kathleen C. Andrews

The effect of administration of methionine on the cystine output of the cystinuric subject has been reported during past years by several investigators with somewhat divergent results. Brand, Cahill, and Harris (1) have reported a marked increase in daily cystine excretion while Andrews and Randall (2), working with a preadolescent cystinuric, found that the administration of the same amounts of methionine as employed by Brand and co-workers produced very little extra cystine. Later experiments by Brand (private communication) on a younger (preadolescent) sister of his former cystinuric subject showed no appreciable increase in cystine output. Lewis, Brown, and White (3) reported, from a similar experiment, an increase in cystine excretion intermediate between the results of Brand and co-workers and those of Andrews and Randall. A recent report by Hess and Sullivan (4) indicates that in their hands the feeding of methionine to a cystinuric produced no increase in cystine excretion.

Since cystinuria is essentially a defect in some biological oxidative mechanism, it appeared possible that the positive results obtained in some cases with methionine, cysteine, etc., might be due to the introduction into the organism of large amounts of a reducing substance. The ease with which methionine is demethylated makes its use practically equivalent to that of a corresponding amount of homocysteine. Hence the administration, instead, of large doses of ascorbic acid (Vitamin C) appeared to be a means of testing the hypothesis. Moreover, the fact that we have yet available the same cystinuric subject (now aged 16) as that used three years earlier by Andrews and Randall (2) makes a repetition of methionine administration a matter of interest.

Table I shows the results in terms of 24-hour output obtained from an experiment extending over about 5 weeks. During this time the subject was on a rigidly controlled diet as indicated by the total nitrogen figures.

* An abstract of this paper was published in the Proceedings of the American Society of Biological Chemists, March, 1938.

TABLE I

Effect of administration of cystine, methionine and ascorbic acid on the urinary output of cystine in the cystinuric

DATE	VOL.	TOTAL N	CREATININE	TOTAL S	SO_4—S	PER CENT OXIDATION	CYSTINE S	CuCl-S	α X VOL.	ASCORBIC ACID
	ml.	*gm.*	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*ml.*	*mg.*
4/8	920	7.12	0.80	0.457	0.254	55.6	0.125		247	0.6
4/9	790	7.00	0.76	0.416	0.251	60.3	0.110		273	0.8
4/10[1]	850	7.44	0.79	1.248	0.946	75.8	0.117		302	0.8
4/11	1,280	7.32	0.88	0.622	0.444	71.4	0.131		447	1.1
4/12	820	6.91	0.79	0.577	0.400	69.4	0.122		221	0.6
4/13	870	6.94	0.74	0.425	0.267	62.8	0.120		201	0.5
4/14	850	7.00	0.74	0.492	0.287	58.3	0.108		175	0.4
4/15	960	7.26	0.76	0.450	0.296	65.8	0.126		290	0.6
4/16	700	6.86	0.78	0.446	0.275	61.7	0.111		300	0.6
4/17[2]	730	6.97	0.79	0.426	0.261	61.3	0.127	0.150	337	0.7
4/18[3]	3,660	8.45	0.87	0.640	0.398	62.2	0.051	0.201	650	690.0
4/19	1,200	7.02	0.83	0.498	0.298	59.8	0	0.180	480	1,428.0
4/20	870	6.98	0.80	0.443	0.261	58.9	0.079	0.132	391	62.2
4/21	840	7.18	0.83	0.472	0.284	60.2	0.116	0.143	352	13.0
4/22	1,000	7.02	0.74	0.426	0.254	59.6	0.128	0.144	300	1.2
4/23	880	6.94	0.78	0.473	0.289	61.1	0.124	0.148	354	0.6
4/24	800	6.98	0.77	0.446	0.273	61.2	0.131	0.149	377	0.8
4/25[4]	2,520	7.72	0.94	0.590	0.333	56.4	0.182	0.218	630	2.3
4/26[4]	3,920	8.38	0.97	0.601	0.341	56.7	0.197	0.240	676	2.3
4/27	700	6.40	0.72	0.391	0.246	62.9	0.114	0.144	287	1.2
4/28	1,060	6.74	0.77	0.385	0.227	59.0	0.118	0.142	350	1.9
4/29	1,200	6.95	0.79	0.384	0.232	60.5	0.124	0.145	300	0.6
4/30	850	6.91	0.79	0.403	0.241	59.8	0.116	0.151	383	0.5
5/1[5]	890	7.02	0.82	0.400	0.248	62.0	0.048	0.147	395	1.2
5/2[6]	980	6.88	0.78	0.435	0.254	58.4	0	0.163	364	871.0
5/3	1,000	7.16	0.85	0.452	0.279	61.7	0	0.165	330	2,380.0
5/4	800	7.10	0.81	0.399	0.237	59.4	0.040	0.142	316	44.0
5/5	900	6.93	0.78	0.391	0.239	61.1	0.065	0.140	310	30.1
5/6	860	7.01	0.83	0.388	0.243	62.6	0.116	0.131	296	39.5
5/7	890	6.90	0.76	0.425	0.252	59.3	0.128	0.150	312	22.8
5/8[7]	810	7.08	0.78	0.423	0.264	62.4	0.114	0.142	320	0.8
5/9[8]	930	7.22	0.79	0.692	0.488	70.5	0.13	0.168	373	1.2
5/10	990	7.36	0.77	1.302	0.970	74.3	0.156	0.192	445	1.3
5/11	840	7.18	0.73	0.578	0.385	66.6	0.106	0.151	358	0.9
5/12	800	6.92	0.78	0.431	0.283	65.6	0.120	0.144	312	1.1
5/13	940	7.03	0.81	0.412	0.249	60.5	0.104	0.138	342	1.3

[1] 5 gm. cystine fed, 8 A.M.

[2] Ascorbic acid fed: 1 gm. at 8 A.M. and 1 gm. at 1 P.M.

[3] Ascorbic acid fed: 1 gm. at 8 A.M., 1 gm. at 1 P.M. and 1 gm. at 7 P.M.

[4] Water drinking for production of diuresis.

[5] Ascorbic acid fed: 2 gm. at 8 A.M., 2 gm. at 1 P.M. and 1 gm. at 7 P.M.

[6] Ascorbic acid fed: 2 gm. at 8 A.M., 2 gm. at 1 P.M. and 2 gm. at 7 P.M.

[7] 2 gm. dl-methionine fed, 7 A.M.

[8] 5 gm. dl-methionine fed, 7 A.M.

A preliminary administration of 5 gm. cystine (April 10) was used to demonstrate again the extent of conversion of cystine to inorganic sulfate. After several days, ascorbic acid was administered by mouth, 2 gm. on April 17 and 3 gm. on April 18. This dosage seemingly produced excessive thirst with the result that the large volume of urine excreted on April 18 was probably more responsible for any increases than was the ascorbic acid. To ascertain this a water drinking experiment was later inserted (April 25 and April 26) while on subsequent administration of ascorbic acid the water intake was controlled. On May 1 and May 2 there were fed, respectively, 5 and 6 gm. ascorbic acid. These doses were divided into three parts and fed before each of the three meals. On May 8 and May 9, 2 and 5 gm. methionine, respectively, were fed.

Determinations of the usual urinary constituents were made as described in the paper by Andrews and Randall (2). Cystine was determined by the revised Sullivan method as described by Sullivan and Hess (5). However, the large amounts of ascorbic acid excreted after its administration made Sullivan determinations impossible, even by the modified procedure, and it was therefore found necessary to resort to the cuprous mercaptid separation of Russouw and Wilken-Jorden (6). The figures for cystine sulfur obtained by this method are listed under the column headed "CuCl–S". The results of these attempts to apply the Sullivan method to urines high in ascorbic acid confirm our previous results (7) on the inhibitory effect of ascorbic acid on the production of color in the Sullivan method. Even prolonged aeration of these urines, while it reduced the ascorbic acid content as indicated by indophenol titration, did not make possible the development of the Sullivan color. This supports our previous findings as to the presence of reducing agents other than ascorbic acid, which interfere with the Sullivan method, in urine.

The column headed "α x ml." provides further evidence as to the excretion of cystine. Since the optical activity of a cystinuric urine is largely the result of its cystine content, an arbitrary measure of this is provided by the product of the 24-hour volume of urine and the observed activity (α). In this case all readings were made in a 2 dm. tube. This product, while an arbitrary number, serves as a means of detecting marked changes in the total cystine output.

Ascorbic acid was determined by standard indophenol titration as described by Bennett and Tarbert (8).

DISCUSSION

Taking the average value for the daily output of cystine sulfur under normal conditions as 0.120 gm. the effect of methionine administration was to raise this to a maximum of 0.156 gm., a 30% increase. Based on the daily output of "CuCl–S" as determined by the method of Russouw and Wilken-Jorden, an increase from a daily average of 0.142 gm. to a maximum of 0.192 gm., a 35% increase is indicated. This result approaches more nearly that obtained by Brand and co-workers than was the case when our subject was preadolescent but the increase in cystine output is still far less marked than that reported by them.

It should furthermore be emphasized that the diuresis experiment of April 25 and April 26 produced a greater increase in cystine output (47% increase by the Sullivan method and 69% by the cuprous chloride method) than that which resulted from methionine administration. Administration of large doses of ascorbic acid produced, in analyses made by the cuprous chloride method, a maximum increase of only about 16% where the water intake was controlled (May 1 to May 3) in contrast to that of 41% when uncontrolled water drinking was permitted.

The order of increase indicated by the product of "α x ml." leads to the same conclusion. The diuresis experiment produced the largest increase in this product (over 100%). Methionine administration produced an increase of about 50% and ascorbic acid about 30%.

The daily urine volume of the subject is obviously a matter of prime importance in the interpretation of such experiments.

CONCLUSIONS

A comparison was made of the effect of diuresis, of methionine administration, and of ascorbic acid administration on the cystine output of a cystinuric subject.

Doses of ascorbic acid up to 6.0 gm. per day produced, with controlled water intake, an increase of only about 16% over the average normal figure.

Doses of methionine, up to 5.0 gm. per day produced a 30 to 35% increase in the cystine output over the normal. This is a considerably greater increase than that obtained three years previously with the same subject when preadolescent.

Water drinking, approximately quadrupling the daily urine volume, increased the cystine output by nearly 50% by the direct Sullivan method and nearly 70% by the cuprous chloride method.

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TECHNIC FOR COLLECTION, ISOLATION AND CULTURE OF CHYTRIDS

By JOHN N. COUCH

By applying the collecting methods used for water molds (Coker, 1923; Harvey, 1925) we have been able to collect numerous Chytridiales and related forms from water and soil. The water with decaying leaves, grass, trash, and mud is put in Petri dishes or other suitable containers and to the soil collections is added charcoal water (used because the spores of aquatic Phycomycetes are very sensitive to distilled H_2O). Charcoal water (Couch, 1931) is prepared as follows: 1 litre distilled H_2O and 1 level teaspoonful of blood or animal charcoal; filter after 1 hour; autoclave. For bait, pollen grains (Zopf, 1887) (*Liquidambar* is particularly good and may be kept dried for years) or boiled pieces of young corn leaves or grass leaves have been used (Karling 1937, 1938, and numerous earlier papers). The pollen grains are picked up on a needle and dusted on the surface of the water; the pieces of leaves are merely dropped in. After being in the dish two or three days, the bait should be examined. If, on first examination, there are no chytrids, fresh leaves should be added, for some chytrids may be slow in appearing. Decolorizing the leaves by boiling in 95% alcohol in a water bath (a process introduced into our laboratory by Miss Berdan, summer 1938) facilitates the study of the chytrids, but such leaves are not so good as those with chlorophyll for the growth and preservation of chytrids. Other chytrids occur on algae and the oogonia and threads of various filamentous aquatic Phycomycetes.

Since only a few chytrids (Zopf 1887, Sparrow 1931, Butler and Humphries 1932, Karling 1937, 1938, etc.) have been isolated in pure fungal culture and none in single spore or single sporangial culture, it seemed worthwhile to try to develop methods for their isolation and culture free from other fungi and if possible free from bacteria. This paper is a preliminary report on the methods used, with a list of the chytrids so isolated and in culture. For the sake of brevity the methods used may be illustrated by chytrids found on pieces of boiled grass leaves.

After one has discovered the presence of chytrids on a piece of grass in the original dish, the piece is transferred to a sterile Petri dish and washed thoroughly with a strong stream of water from a wash bottle, holding the leaf meanwhile with forceps and tilting the dish so that the trash and many of the undesirable organisms are removed.

Sometimes one may secure a single chytrid species on one piece of grass but more frequently several species occur, as well as leaf-inhabiting forms of *Pythium* and other water molds.

Changing the water will hasten spore discharge of the chytrids as well as the other fungi, hence it is well to have culture dishes, tools, binocular microscope, and everything needed ready before the leaf is removed from the original dish. An examination with the compound microscope (low power) will show what material is worth isolating and enable one to slash off and discard large areas of the leaf infected with *Pythium*, etc.

We have found the following methods most effective in isolating chytrids in pure fungal culture, i.e. free from all other organisms except bacteria and in some cases free from bacteria: (1) isolation in water of a single sporangium or (2) isolation on agar of a single sporangium; (3) isolation of spores from a single sporangium on slide; (4) isolation of single spore in a capillary tube or (5) isolation of single spore on agar or (6) isolation of a single thread or several threads on agar.

With method (1) the procedure may be as follows: Place leaf with chytrids in a drop of water in a Petri dish and in another drop of water about one cm. away in the same dish put a new piece of sterile leaf. Then under a wide field binocular dissecting microscope (×40–×100) with sharp, smooth, steel needles dissect out a small piece of leaf to which only one sporangium is attached. Transfer this to a small drop of water in the same dish and examine under compound microscope to be sure only one sporangium and no spores are present. The fragment of leaf with the sporangium may now be transferred to the fresh piece of leaf. All transfers up to this stage are made from drop to drop in the same dish, because if the delicate chytrid were transferred from one dish to another, it might dry up in the operation. After the chytrid sporangium is on the large, moist leaf, the latter is transferred to a drop of charcoal water in a fresh sterile dish and other drops of water are added to the floor and ceiling of the dish to prevent desiccation. In this operation many of the single sporangia are injured in transfer. Hence it is advisable to make large numbers of transfers.

If one is lucky, each isolated sporangium will form zoospores which

will infect the new leaf, thus establishing the chytrid in pure culture save for the presence of bacteria.

The above technic is useful only if the sporangia are large. A more useful technic (2), particularly with small sporangia, and where several species are mixed and are discharging spores simultaneously, is as follows. An infected leaf is transferred after washing to the surface of a 3% agar plate (see formula below). The desired chytrid sporangium is now dissected out from the leaf tissue and dragged along on the surface of the agar to free it from spores, bacteria, etc. After examining under the compound microscope to make sure that only one chytrid sporangium is now present, it is cut out with a little cube of agar and transferred to a fresh Petri dish in a drop of water with a fresh piece of leaf, other drops of water being added to the bottom of the dish to prevent desiccation. This is a very useful technic because it enables one to transfer nothing but the sporangium and its rhizoids. We have used a slight modification of this method by tearing the leaf tissue apart on the surface of the agar and spreading it out. If water is present, some of the sporangia may discharge their spores on the surface of the agar. The spores may then germinate in contact with the leaf, sending their rhizoids into the agar. It is possible then to remove the leaf, wash the surface of the agar with water from the wash bottle and then to dissect out one sporangium from the agar surface.

If the rhizoidal system is very complex and the spores of other fungi abundant, the method just described may be unsatisfactory, in which case the following method (3) is useful. A single sporangium about ready to discharge spores is isolated by method 1 or 2 and put on a sterile slide in a drop of water and kept under observation so that one may determine just when the spores emerge. The moment this happens some of the spores are drawn up in a capillary tube and blown out in a drop of water with a piece of sterile leaf in a fresh Petri dish. If ordinary care is used one may secure cultures by this technic descended from a few spores. It is possible so to perfect this method that one can, with a little practice and skill, make single spore cultures. This may be done as follows: (4) By using a very fine capillary tube and picking up only a few spores, then diluting the spores by mixing in another drop of water and so on by further dilutions, it is possible finally to get only one or two spores in the capillary tube. If the tube were clean to begin with, it may be examined on the surface of the Petri dish to determine how many spores it contains. If only one spore is present, the tube is then transfered to a drop of water containing a piece of leaf. If it

contains two or even several spores, it is possible to break the tube in such a way that one can separate a single spore from the others.

None of the methods so far described separates the chytrids from bacteria, although it is possible with great precaution to keep the cultures relatively free from them. It is possible, however, to isolate some of the chytrids in pure culture. So far we have developed two methods for doing this. The easier, if the spores will germinate on agar, is by the isolation of a single spore on agar (5). After much experimenting with spore germination on nutrient agars, we have found several which are useful in this work.

1. Plain agar 3% (the agar shreds should be washed over night in several changes of water to free from trash).
2. Agar No. 12 (Leitner's agar) 2% agar and 0.004% peptone (meat).
3. Agar No. 13 (Foust's agar) 2% agar and 0.15% maltose and 0.004% peptone (meat).
4. Corn meal agar (use 2–4 heaping teaspoons full to 1 litre water, depending upon strength desired. Heat gently in water bath, temperature about 60°, 1 hour. Filter. Add water to make 1 litre. Agar 2%).

The spores seem to germinate better on plain agar or agar with very small amounts of nutrient material. Before taking time to spread the spores carefully on agar it is worth while to drop a few on the medium used to determine whether or not the spores go to pieces or settle down and encyst. Naturally if the spores are plasmolized by the nutrient agar it is a waste of time to go further with that particular medium. Spores to be isolated should be as free from bacteria as possible. Hence, it is well to isolate one or a few sporangia about to discharge spores in a drop of water on a sterile slide. However, where only a few spores are available it is possible to pick them up with a platinum loop or a small pipette directly from the original dish. The essential part of this technic is to spread the spores so well on the surface of the agar that some of the spores will be completely separated from the bacteria and other organisms. A successful spreading requires a firm agar (2–3% agar) and a steady hand. The spores are picked up with a platinum loop and the loop dragged along the surface of the plate in a straight line. Several east-west lines may be made and then another group of north-south ones. It is unnecessary to mark these lines for the bacterial colonies will make the lines quite evident. If the laboratory is clean and free from spores of *Penicillium*, etc., it is possible to do this spreading with the uncovered dish on the table. However, if the air is dusty, the dish should be held at an angle with the agar surface down. If the

spores germinate at all they will germinate within 12 to 24 hours or in even less time. After 12 hours the plate should be examined under the binocular dissecting microscope (×40–×100). If the spores have been properly spread, one may now cut out a tiny block of agar with a single

TABLE I

NAME	DATE	FOUND ON	ISOLATED BY	CULTURED ON	PRESENT CONDITION
Rhizidiomyces apophysatus	1932	Ao	1, 2, 5	13, Ao, p.	pfc
Catenaria-like without rhizoids	1932	p	1, 5	p, Ao, 13 etc.	pc
Rozella septigena	1938	A. c.	Host threads and par. spores	Host	pfc + host
Woronina polycistis	1938	A. f.	Host threads and par. spores	Host	pc + host; pfc + host
Rhizophidium carpophilum	1934	Ao	5	h, 13, p, cl	pfc
Rhizophidium multiporum	1937	Ao	5	h, 13, p, cl	pfc
Rhizophidium n. sp.	1937	Ao	5	h, 13, p, cl	pfc
Rhizophlyctis rosea	1938	gl in soil collection	1, 5	cn, cl, gl, fp	pfc
Rhizophlyctis n. sp.	1938	gl in soil collection	1, 2	cl, gl, fp	pfc
Nowakowskiella elegans	1938	gl in soil collection	2	cl, gl, fp	pfc
Siphonochytrium n. gen.	1938	gl in soil collection	4, 5	13, gl, cl, fp	pfc
Cladochytrium replicatum	1938	gl in H_2O collection	6	Various agars	pc
Lagenidium giganteum	1933	Mosquito larva	5	Various agars	pc
Myzocytium sp.	1938	Closterium	2	Various agars	pc
Resticularia (?)	1938	Oscillatoria	6	Various agars	pc

sporangium descended from a single spore. The cutting out operation requires a very fine tool. For this we use a tiny chisel made by sharpening down a steel needle under the microscope. Individual sporangia may be transferred to a drop of water in a sterile Petri dish on a piece of

leaf. Such a culture descended from a single spore may be kept free from bacteria for a few generations in water cultures. In some chytrids the sporangia mature and discharge their spores on agar. It is possible, though exceedingly tedious, to keep such cultures pure, growing on agar and free from bacteria. The labor involved, however, is excessive and we have carried such cultures on agar for only a few generations.

In some of the polycentric chytrids as *Cladochytrium replicatum* the spores germinated on agar to produce a distinct mycelium. It is therefore possible to isolate this species by cutting out a single thread or several threads. This method (6), however, is useless with the monocentric chytrids.

The following table summarizes some of our results in trying to culture chytrids and related forms. In each case the name of the chytrid is given, the host or substratum on which it was found, the method, 1–6 (see p. 209 above), by which it was isolated, the substrata used in its culture and its present condition. The agars used are referred to by letters or number, i.e. agar no. 12, 12; agar no. 13, 13; and corn meal agar, cm. Other substrata or hosts are also abbreviated: oogonia of *Achlya*, Ao; *Achlya caroliniana*, A. c.; *Achlya flagellata*, A. f.; pollen grains, p; boiled corn leaves, cl; boiled grass leaves, gl; boiled filter paper, fp. In the last column the present condition of the fungus is indicated: Pc, pure culture free from other organisms; pfc, pure culture of fungus but with bacteria. Only the species now in culture are listed in table I.

SUMMARY

Methods are described by which 15 representative species from all the larger groups of the Chytridiales and related forms have been isolated and cultured, some for as long as seven years. All of these forms have been isolated by a single sporangium, or a single spore, or pure threads and hence the cultures descended from such isolations are known to consist of a single fungal strain. Several forms are in pure culture on agar, the others are in single fungal culture but with bacteria. In addition to the usual substrata as boiled leaves and pollen, four different species (see table fp) have been grown on boiled filter paper in water. In such cultures bacteria have been present. Indeed, bacteria seem to be necessary for their growth, since spores put on sterile filter paper in water failed to grow. Four species of monocentric chytrids, *Rhizidiomyces apophysatus*, *Rhizophidium carpophilum*, *R. multiporum* and *R.* n. sp. have been carried on agar in pure culture

through several generations. This is possibly due to the fact that in these forms the sporangia mature and spores emerge on agar and thus fresh spores may be picked up and spread on fresh agar plates. In the greater number of chytrids tested the spores germinated and grew best on agars with small amounts of nutrients or even on plain agar.

In the present report only the forms which could be cultured are considered. Several other species were tested but failed to grow.

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